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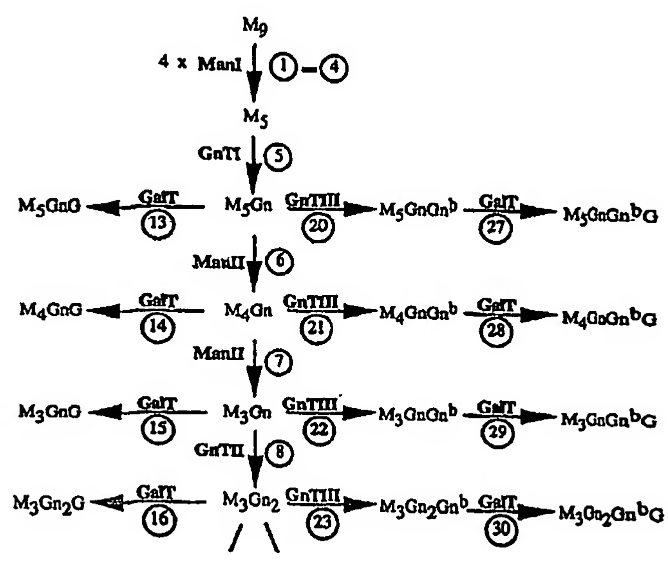
(54) Title: GNTIII EXPRESSION IN PLANTS



(57) Abstract: The invention relates to the field of glycoprotein processing in transgenic plants used as cost efficient and contamination safe factories for the production of recombinant biopharmaceutical proteins or pharmaceutical compositions comprising these glycoproteins. The invention provides a plant comprising a functional mammalian enzyme providing mammalian GnTIII that is normally not present in plants, said plant additionally comprising at least a second mammalian protein or functional fragment thereof that is normally not present in plants.

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GNTIII EXPRESSION IN PLANTS

FIELD OF THE INVENTION

The invention relates to expression of a mammalian N-acetylglucosaminyl-transferase III (GnTIII) enzyme in plants and its use in producing glycoproteins with bisected oligosaccharides and increased amount of terminal G1cNAc residues. The invention further relates to a hybrid protein comprising the catalytic site of GnTIII and transmembrane domain of Golgi apparatus and/or endoplasmic reticulum (ER) protein or modified GNTIII comprising ER retention signals and its use in producing glycoproteins with oligosacchararides that lack immunogenic xylose and fucose residues.

BACKGROUND OF THE INVENTION

N-Acetylglucosaminyltransferases (GlcNAc-transferases) are "branching" enzymes that add an Nacetylglucosamine (G1cNAc) residue to one of the mannoses of the trimannosyl core structure of typical Nlinked glycans. At least six G1cNAc-transferases are known with little or no sequence homology. Besides different protein structures, these G1cNActransferases also have different enzymatic properties and substrate specificity. All are typical type II transmembrane proteins with a cytoplasmic domain, a transmembrane anchor and an extracellular stem region with catalytic domain.

A remarkable G1cNAc-transferase is G1cNAc-transferase III (GnTIII). GnTIII, also known as UDP-Nacetylglucosamine:β-D-mannoside β(1,4)-N-acetylglucosaminyl-transferase III (EC 2.4.1.144), inserts bisecting G1cNAc residues in complex-type N-linked glycans of cellular glycoproteins (for a review see Taniguchi, *et al.*, "A glycomic approach to the identification and characterization of glycoprotein function in cells transfected with glycosyltransferase genes" *Proteomics* 1:239247, 2001). GnTIII adds the G1cNAc through a β(1,4) linkage to the β-linked mannose of the trimannosyl core structure of the N-linked glycan. GnTIII was first identified in hen oviduct (Narasimhan S., "Control of glycoprotein synthesis. UDP-G1cNAc:glycopeptide β 4-Nacetylglucosaminyltransferase III, an enzyme in hen oviduct which adds G1cNAc in β14 linkage to the β-linked mannose of the trimannosyl core of N-glycosyl oligosaccharides" *The Journal of Biological Chemistry* 257:10235-10242, 1982) but a high level of activity has also been reported in various types of rat hepatomas, human serum, liver and hepatoma tissues of patients with hepatomas and liver cirrhosis (Ishibashi, *et al.*, "N-acetylglucosaminyltransferase III in human serum and liver and hepatoma tissues: increased activity in liver cirrhos and hepatoma patients" *Clinical Chimica Acta* 185:325, 1989; Narishimhan, *et al.*, "Expression of N-acetylglucosaminyltransferase III in hepatic nodules during rat liver carcinogenesis promoted by orotic acid" *Journal of Biological Chemistry* 263:1273-1281, 1988; Nishikawa, *et al.* "Determination of N-acetylglucosaminyltransferases III, IV and V in normal and hepatoma tissues of rats" *Biochimica et Biophysica Acta* 1035:313-318, 1990; Pascale, *et al.*, "Expression of N-acetylglucosaminyltransferase III in hepatic nodules generated by different models of rat liver carcinogenesis" *Carcinogenesis* 10:961964, 1989). Bisected oligosccharides on glycoproteins have

been implicated in antibody-dependent cellular cytotoxicity (ADCC). ADCC is a lytic attack on antibody-targeted cells and is triggered upon binding of lymphocyte receptors to the constant region (Fc) of antibodies. Controlled expression of GnTIII in recombinant Chinese Hamster Ovary (CHO) production cell lines that lack GnTIII activity resulted in antibodies with bisected oligosaccharides with optimized ADCC activity (Davies, *et al.*, "Expression of GnTIII in a recombinant anti-CD20 CHO production cell line: expression of antibodies with altered glycoforms leads to an increase in ADCC through higher affinity for FcγRIII" *Biotechnology and Bioengineering* 74:288-294, 2001; Umana, *et al.*, "Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity" *Nature Biotechnology* 17:176-180, 1999). The ADCC activity correlated well with the level of Fc region-associated bisected complex oligosaccharides present on the recombinant antibody (Umana, *et al.*, "Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity" *Nature Biotechnology* 17:176-180, 1999). Bisecting GlcNAc residues resulting from GnTIII activity affect the conformation of the sugar chains in such a way that other glycosyltransferases such as GlcNAc-transferase II and α1,6-fucosyltransferase, but not β(1,4)-galactosyltransferase, can no longer act (Tanigichi, *et al.*, 2001). Overexpression of GnTIII in CHO cells is lethal.

In contrast to typical mammalian production cell lines such as CHO cells, transgenic plants are generally recognized as a safe production system for therapeutic proteins. Plant glycoproteins, however, differ in oligosaccharide structure with those from mammals in several aspects. They lack terminal galactose and sialic acid, have an additional core xylose and differently linked core fucose (α-1,3) instead of (α-1,6). Like CHO and other pharmaceutical production cell lines they also completely lack bisected oligosaccharides. Plants have the capacity to generate the common core structure, GN2M3GN2 but predominantly M3 GN2 variants are found, indicating removal of terminal GN by hexosaminidases.

Biogenesis of N-linked glycans begins with the synthesis of a lipid linked oligosaccharide moiety (Glc3Man9GlcNAc2-) which is transferred *en bloc* to the nascent polypeptide chain in the endoplasmic reticulum (ER). Through a series of trimming reactions by exoglycosidases in the ER and cis-Golgi compartments the so-called "high mannose" (Man9GlcNAc2 to Man5GlcNAc2) glycans are formed. Subsequently, the formation of complex type glycans starts with the transfer of the first GlcNAc onto Man5GlCNAC2 by GnTI and further trimming by mannosidase II (Mann) to form GlcNAcMan3 GlcNAc2. Complex glycan biosynthesis continues while the glycoprotein is progressing through the secretory pathway with the transfer in the Golgi apparatus of the second GlcNAc residue by GnTII as well as other monosaccharide residues onto the GlcNAcMan3GlcNAc2 under the action of several other glycosyl transferases. Plants and mammals differ with respect to the formation of complex glycans. In plants, complex glycans are characterized by the presence of β(1,2)-xylose residues linked to the Man-3 and/or an α(1,3)-fucose residue linked to GlcNAc1, instead of an α(1,6)-fucose residue linked to the GlcNAc-1 (Lerouge, P., *et al.*, "N-glycoprotein biosynthesis in plants: recent developments and future trends" *Plant Mol Biol* 38:31-48, 1998). Genes encoding the corresponding xylosyl (XylT) and fucosyl (FucT) transferases have been

isolated (Strasser R, "Molecular cloning and functional expression of β l, 2-xylosyltransferase cDNA from *Arabidopsis thaliana*" *FEBS Lett.* 472:105-8, 2000; Leiter, H., *et al*, "Purification, cDNA cloning, and expression of GDP-L-Fuc:Asn-linked GlcNAc α 1,3-fucosyltransferase from mung beans" *J Biol Chem.* 274:21830, 1999). Xylose and fucose epitopes are known to be highly immunogenic and possibly allergenic which may pose a problem when plant are used for the production of therapeutic glycoproteins. Moreover, blood serum of many allergy patients contains IgE directed against these epitopes which make particularly these patients at risk to treatments with xylose and fucose containing recombinant proteins. In addition, this carbohydrate directed IgE in sera might cause false positive reaction in *in vitro* tests using plant extracts since there is evidence that these carbohydrate specific IgE's are not relevant for the allergenic reaction. Plants do not possess β(1,4)galactosyltransferases nor α(2,6)sialyltransferases and consequently plant glycans lack the β(1,4)galactose and terminal α(2,6)NeuAc residues often found on mammalian glycans (Vitale and Chrispeels, "Transient N-acetylglucosamine in the biosynthesis of phytohemagglutinin: attachment in the Golgi apparatus and removal in protein bodies" *J Cell Biol* 99:133-140, 1984; Lerouge, P., *et al.*, "N-glycoprotein biosynthesis in plants: recent developments and future trends" *Plant Mol Biol* 38:31-48, 1998).

The final glycan structures are not only determined by the mere presence of enzymes involved in their biosynthesis but to a large extend by the specific sequence of the various enzymatic reactions. The latter is controlled by discrete sequestering and relative position of these enzymes throughout the ER and Golgi, which is mediated by the interaction of determinants of the transferase and specific characteristics of the sub-Golgi compartment for which the transferase is destined. A number of studies using hybrid, molecules have identified that the transmembrane domains of several glycosyltransferases play a central role in their sub-Golgi sorting (Grabenhorst E., *et.al.*, *J. Biol. Chem.* 274:36107-36116, 1999; Colley, K., *Glycobiology*.7:1-13, 1997, Munro, S., *Trends Cell Biol.* 8:11-15, 1998; Gleeson P.A., *Histochem. Cell Biol.* 109:517-532, 1998).

Similar to mammalian production cell lines used in pharmaceutical industry, glycoproteins produced in plants lack GnTIII activity. Plants not only lack GnTIII activity but are completely devoid of GnTIII-like sequences. In addition, plants also lack GnTIV, GnTV ands GnTVI sequences and moreover, sialic acid residues. (For an overview of the major glycosylation attributes of commonly used cell expression systems including plants see, Jenkins, *et al.*, "Getting the glycosylation right: implications for the biotechnology industry" *Nature Biotechnology* 14:975-979, 1996). Nevertheless, plants are a very potent production system. Plants are generally accepted as safe and are free of particles infectious to humans. Plant production is easy scalable and N-linked glycosylation can be controlled (Bakker, *et al.*, "Galactose-extended glycans of antibodies produced by transgenic plants" *Proc. Nat. Acad. Sci. USA* 98:2899-2904, 2001).

Transgenic tobacco plants that produce galactosylated recombinant monoclonal antibodies (Mabs) upon introduction of the human gene for β(1,4)-galactosyltransferase have been reported (hGalT; Bakker, *et al.*, "Galactose-extended glycans of antibodies produced by transgenic plants" *Proc. Nat. Acad. Sci. USA* 98:2899-2904, 2001; WO01/31044 and WO01/31045).

Therapeutic glycoproteins can be improved by altering their glycosylation pattern (Davies, *et al.*, "Expression of GnTIII in a recombinant anti-CD20 CHO production cell line: expression of antibodies with altered glycoforms leads to an increase in ADCC through higher affinity for FcγRIII" *Biotechnology and Bioengineering* 74:288-294, 2001; Umana, *et al.*, "Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity" *Nature Biotechnology* 17:176-180, 1999; Fukuta, *et al.*, "Remodeling of sugar chain structures of human interferon-γ " *Glycobiology* 10:421-430, 2000; Misaizu, *et al.*, "Role of antennary structure of N-linked sugar chains in renal handling of recombinant human erythropoietin" *Blood* 86:4097-4104, 1995; Sburlati, *et al.*, "Synthesis of bisected glycoforms of recombinant IFN-β by overexpression of β-1,4-N-acetylglucosaminyl-tranferase III in Chinese Hamster Ovary cells" *Biotechnology Prog.* 14:189-192, 1998). Higher oligosaccharide antennarity of EPO, for example, leads to increased *in vivo* activity due to reduced kidney filtration (Misaizu, *et al.*, "Role of antennary structure of N-linked sugar chains in renal handling of recombinant human erythropoietin" *Blood* 86:4097-4104, 1995). Biosynthesis of such superior glycoforms can be achieved with the "standard" glycosylation machinery of normal production cell lines by two methodologies. The first is by enriching specific glycoforms during purification and the second is by introducing mutations in the polypeptide chain. The latter makes it possible to shift the glycosylation site within the glycoprotein resulting in different glycosylation patterns as the result of differences in accessibility. A complementary route is through genetic engineering of the production cell line itself. New glycosylation patterns can be obtained through expression of glycosyltransferase and glycosidase genes in production cell lines. These genes code for enzymes that either add or remove specific saccharides to and from the glycan of cellular glycoproteins. Several glycosyltransferase genes have been introduced in CHO cells to manipulate glycoform biosynthesis. One of them is GnTIII. Glycosyltransferase GnTIII is involved in branching of the N-linked glycan and results in bisecting GlcNAc residues. CHO cells and other production cell lines typically lack GnTIII activity (Stanley, P. and C.A. Campbell, "A dominant mutation to ricin resistance in chinese hamster ovary cells induces UDP-GIcNAc: glycopeptide β-4-N-acetylglucosaminyl-transferase III activity" *Journal of Biological Chemistry* 261:13370-13378, 1984). Expression of GnTIII in CHO resulted in bisected complex oligosaccharides as expected but overexpression resulted in growth inhibition and was toxic to cells. Similarly, overexpression of GnTV, another glycosyltransferase that introduces triantennary sugar chains, also resulted in growth inhibition suggesting that this may be a general feature of glycosyltransferase overexpression (Umana, *et al.*, "Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity" *Nature Biotechnology* 17:176-180, 1999).

Therefore, there is a need to provide a means for producing glycoprotein in plants with human compatible non-immunogenic bisecting oligosaccharides.

SUMMARY OF THE INVENTION

The invention relates to expression of a mammalian N-acetylglucosaminyl-transferase III (GnTIII) enzyme in plants and its use in producing glycoproteins with bisected oligosaccharides and

increased amount of terminal GlcNAc residues. The invention further relates to a hybrid protein comprising the catalytic site of GnTIII and transmembrane domain of Golgi apparatus and/or endoplasmic reticulum (ER) protein or modified GNTIII comprising ER retention signals and its use in producing glycoproteins with oligosaccharides that lack immunogenic xylose and fucose residues.

In one embodiment, the present invention contemplates a plant host system comprising or expressing a mammalian UDP-Nacetylglucosamine:(β-D-mannoside β(1,4)-Nacetylglucosaminyltransferase (GnTIII) enzyme (nucleotide sequence: SEQ ID NO.: 1, Genbank I.D. number AL022312 (Dunham, I., *et al.*, *Nature* 402:489-495, 1999); protein sequence: SEQ ID NO.: 2, Genbank I.D. number Q09327), wherein said GnTIII inserts bisecting Nacetyl glucosamine (GlcNAc) residues in complex-type N-linked glycans of a glycoprotein present in said plant host system

In a specific embodiment of the invention, the plant host system further comprises a heterologous glycoprotein or functional fragment thereof comprising bisected oligosaccharide, particularly galactose residues. The GnTIII inserts bisecting N-GlcNAc residues onto said heterologous glycoprotein.

In one embodiment, the present invention contemplates to a method for obtaining a plant host system expressing a heterologous glycoprotein comprising bisecting oligosaccharides. In one embodiment, the method comprises crossing a plant expressing a heterologous glycoprotein with a plant expressing said GnTIII, harvesting progeny from said crossing and selecting a desired progeny plant expressing said heterologous glycoprotein and expressing mammalian GnTIII. Alternatively, said plant host system may be obtained by introducing into a plant or portion thereof a nucleic acid encoding said mammalian GnTIII and a nucleic acid encoding said heterologous glycoprotein and isolating a plant or portion thereof expressing said heterologous glycoprotein and expressing mammalian GnTIII that is normally not present in plants. Furthermore, the invention is directed to a method for obtaining said heterologous glycoprotein from said plant comprising obtaining a plant host system using either of the procedures described above and further isolating said heterologous glycoprotein.

In another embodiment, it is contemplated that the plant host system of the present invention further comprises a functional mammalian enzyme providing N-glycan biosynthesis that is normally not present in plants thereby, for example, providing the capacity to extend an N-linked glycan by the addition of a galactose as described in WO 01 /21045 (herein incorporated by reference). In another embodiment, the present invention further contemplates a plant host system, wherein said plant host system comprises crossing a plant, said plant comprising a functional protein such as a transporter protein or a enzyme (*e.g.*, a mammalian protein) or functional fragment thereof wherein said protein provides N-glycan biosynthesis, with a plant comprising said mammalian GnTIII. In another embodiment, the present invention contemplates harvesting the progeny from said crossing and selecting a desired progeny plant expressing said functional protein such as, for example, a transporter protein or enzyme or functional fragment thereof. In yet another embodiment of the

present invention, it is contemplated that the expressed protein provides N-glycan biosynthesis and the mammalian GnTIII. In still yet another embodiment, the present invention contemplates a plant host system, wherein a nucleic acid encoding the GnTIII and a nucleic acid encoding a functional protein (for example, a transporter or an enzyme [*e.g.*, mammalian] or functional fragment thereof) providing N-glycan biosynthesis and isolating said plant or portion thereof expressing the functional protein or functional fragment thereof providing N-glycan biosynthesis and said mammalian GnTIII. Although the present invention is not limited to any particular theory or mechanism, it is believed that such a combination increases galactosylation of a heterologous glycoprotein. Additionally, in one embodiment, it is contemplated that GnTIII and other proteins providing N-glycosylation such as GalT can also be introduced simultaneously via one transformation vector.

In one embodiment, the present invention contemplates a plant host system comprising expressing said heterologous glycoprotein (wherein, said heterologous glycoprotein has increased galactosylation) and methods for obtaining said plant host cell system and said heterologous glycoprotein. In another embodiment, the plant host cell system may be obtained by either crossing a plant wherein the plant comprises mammalian GnTIII and a functional protein (for example, a transporter or an enzyme [*e.g.*, mammalian] or functional fragment thereof that provides N-glycan biosynthesis not normally found in plants) with a plant comprising a heterologous glycoprotein and, then, selecting said progeny plants. In yet another embodiment, it is contemplated that said heterologous glycoprotein may be obtained by introducing nucleic acid sequences encoding 1) said GnTIII, 2) said functional protein or enzyme providing N-glycan biosysnthesis not normally found in plants and 3) said heterologous glycoprotein into said plant or portion thereof and isolating said plant or portion thereof expressing said nucleic acid sequences. In another embodiement of the present invention, it is contamplated that the heterologous glycoproteins will be isolated or purified from the plant host systems.

In one embodiment of the present invention, a hybrid protein is contemplated, wherein the hybrid protein comprises 1) an isolated hybrid protein comprising a catalytic portion of mammalian GnTIII and 2) a transmembrane portion of a protein from, for example, the endoplamsic reticulum or Golgi apparatus of a eukaryotic cell. In another embodiment, the present invention also contemplates a modified mammalian GnTIII comprising a retention signal such as KDEL for retention of said GnTIII in the ER. In yet another embodiment, the present invention contemplates nucleic acid sequences encoding 1) said hybrid proteins and said modified mammalian GnTIII, 2) vectors comprising said nucleic acid sequences and 3) plant host systems comprising said sequences. In one embodiment, these hybrid proteins and modified GnTIIIs may act to relocalize GnTIII activity in the endoplasmic reticulum (ER) and/or Golgi apparatus. In another embodiment, the present invention contemplates methods for obtaining these hybrid proteins and modified GnTIII proteins by, for example, introducing sequences encoding said hybrid proteins or modified GnTIIIs into a plant or portion thereof. Although the present invention is not limited to any particular theory or mechanism, it is believed that as a result of such relocalization, bisecting GlcNAc is be introduced earlier in the N-glycan biosynthesis sequence of reactions thereby preventing subsequent enzymatic reactions and,

as a consequence, a heterologous protein expressed in a plant host system (for example, the plant host system of the present invention) will lack xylose and fucose and have increased amount of terminal GlcNAc. Accordingly, one embodiment of the present invention contemplates a method to provide a plant host system expressing a heterologous glycoprotein (said plant host system having the capacity to extend an N-linked glycan with galactose) comprising crossing a plant comprising said 1) hybrid protein or said modified GnTIII with a plant comprising said heterologous protein and 2) selecting said desired progeny. In another embodiment, the present invention contemplates introducing into a plant or portion thereof a nucleic acid sequence encoding 1) said modified GnTIII or said hybrid protein and said heterologous glycoprotein and 2) isolating said plant or portion thereof expressing a heterologous glycoprotein with the capacity to extend and N-linked glycan with galactose. In yet another embodiment, the present invention contemplates a method for obtaining said desired heterologous glycoprotein, said method comprising isolating said glycoprotein from said plant or portion thereof.

In one embodiment, the present invention contemplates that the plant-derived glycoprotein or functional fragment thereof may be used for the production of a pharmaceutical composition (for example, an antibody, a hormone, a vaccine antigen, an enzyme, or the like). In another embodiment, the present invention contemplates a pharmaceutical composition comprising a glycoprotein or functional fragment thereof is now also provided.

In one embodiment, the present invention contemplates variants or mutants of GntIII. The terms "variant" and "mutant" when used in reference to a polypeptide refer to an amino acid sequence that differs by one or more amino acids from another, usually related, polypeptide. In another embodiment, the present invention contemplates variants that have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties. One type of conservative amino acid substitutions refers to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine (V) -leucine (L) -isoleucine (I), phenylalanine (F) -tyrosine (Y), lysine (K) -arginine (R), alanine (A) -valine (V), and asparagine (N) -glutamine (Q).

In yet another embodiment, the present invention contemplates variants that have "non-conservative" changes (*e.g.*, replacement of a glycine with a tryptophan). Similar minor variations may also include amino acid deletions or insertions (*i.e.*, additions), or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing biological activity may be found using computer programs well known in the art, for example, DNAStar software. Variants can be tested in functional assays. For both conservative and non-conservative variants, preferred variants have less than 10 %, preferably less than 5 % and,

still more preferably, less than 2 % changes (whether substitutions, deletions, and so on).

In one embodiment, the present invention contemplates a plant host (cell) system, comprising a mammalian UDP-N-acetylglucosamine: β-D mannoside β(1,4)-N-acetylglucosaminyltransferase (GnTIII) enzyme (or portion or variant thereof, wherein said GnTIII inserts bisecting N-acetyl glucosamine (GlcNAc) residues in complex-type N-linked glycans of a glycoprotein present in said plant host system). In another embodiment, the present invention contemplates the plant host, wherein said GnTIII is a human GnTIII. In yet another embodiment, the present invention contemplates the plant host system, wherein said system is a portion of a plant. In yet another embodiment, the present invention contemplates the plant host system, wherein said system is a portion of a plant selected from the group consisting of a cell, leaf, embryo, callus, stem, pericarp, protoplast, root, tuber, kernel, endosperm and embryo. In yet another embodiment, the present invention contemplates the plant host system, wherein said system is a whole plant. In yet another embodiment, the present invention contemplates the plant host system, further comprising a heterologous glycoprotein (or functional fragment thereof). In yet another embodiment, the present invention contemplates the plant host system, wherein said heterologous glycoprotein protein comprises an antibody, or fragment (*e.g.* Fc, Fv, Fab, Fab_2) thereof. In yet another embodiment, the present invention contemplates the plant host system, wherein said heterologous glycoprotein or functional fragment thereof comprises bisected oligosaccharides. In yet another embodiment, the present invention contemplates the plant host system, wherein said heterologous glycoprotein (or functional fragment thereof) comprises bisected glycans with galactose residues. In yet another embodiment, the present invention contemplates the plant host system, wherein said plant is a tobacco plant. In yet another embodiment, the present invention contemplates the plant host system, which further comprises a functional protein selected from a group consisting of a transporter or a (mammalian) enzyme (or functional fragment thereof) providing N-glycan biosynthesis. In yet another embodiment, the present invention contemplates the plant host system, wherein said enzyme is a (human) β-1,4 galactosyltransferase. In yet another embodiment, the present invention contemplates the plant host system, which further comprises a heterologous glycoprotein, having an increased number of galactose residues. In yet another embodiment, the present invention contemplates a plant host system comprising a nucleic acid sequence encoding a mammalian GnTIII protein. In yet another embodiment, the present invention contemplates a plant host system comprising a vector comprising a nucleic acid sequence encoding a mammalian GnTIII protein. In yet another embodiment, the present invention contemplates the plant host, which further comprises a nucleic acid sequence encoding a functional protein selected from a group consisting of a transporter or a (mammalian) enzyme (or functional fragment thereof) providing N-glycan biosynthesis.

In one embodiment, the present invention contemplates a method (for obtaining a plant host system expressing a heterologous glycoprotein having bisected oligosaccharides) comprising a) crossing a plant expressing a heterologous glycoprotein with a, b) harvesting progeny from said crossing and c) selecting a desired progeny plant (expressing said heterologous glycoprotein and

expressing a mammalian GnTIII that is normally not present in plants). In another embodiment, the present invention contemplates this method, wherein said desired progeny plant expresses said heterologous glycoprotein protein having bisected oligosaccharides. In yet another embodiment, the present invention contemplates this method, wherein said plant host system is a transgenic plant.

In one embodiment, the present invention contemplates a method for obtaining a heterologous glycoprotein having bisected oligosaccharides comprising a) introducing a nucleic acid sequence encoding GnTIII that is normally not present in plant into a plant host system and a nucleic acid sequence encoding a heterologous glycoprotein and b) isolating said heterologous glycoprotein. In another embodiment, the present invention contemplates this method, wherein said nucleic acid sequences are introduced into a plant cell and said plant cell is regenerated into a plant. In yet another embodiment, the present invention contemplates the same method, wherein said nucleic acid sequences are introduced into a plant host system by transforming said plant host system with a vector comprising a acid sequence encoding GnTIII that is normally not present in plant into a plant and a nucleic acid sequence encoding a heterologous glycopraxein. In yet another embodiment, the present invention contemplates the method, wherein said nucleic acid sequences are introduced into a plant host system by transforming said plant host system with a vector comprising a nucleic acid sequence encoding GnTIII that is normally not present in plant into a plant and a nucleic acid sequence encoding a heterologous glycoprotein. In yet another embodiment, the present invention contemplates the method, wherein said nucleic acid sequences are introduced into a plant host system by transforming said plant with a vector comprising a nucleic acid sequence encoding GnTIII that is normally not present in plant into a plant host system and vector comprising a nucleic acid sequence encoding a heterologous glycoprotein. In yet another embodiment, the present invention contemplates a method for obtaining a heterologous glycoprotein having bisected oligosaccharides comprising cultivating the regenerated plant.

In one embodiment, the present invention contemplates a method for obtaining a desired glycoprotein (or functional fragment thereof) comprising a) cultivating the plant host system (until said plant has reached a harvestable stage) and b) harvesting said plant (and fractionating to obtain fractionated plant material and c) at least partly isolating said glycoprotein from said fractionated plant material). In another embodiment, the present invention contemplates a plant obtainable by the contemplated method.

In one embodiment, the present invention contemplates A method for obtaining a plant host system comprising a functional protein selected from a group consisting of a transporter or a (mammalian) enzyme or functional fragment thereof providing N-glycan biosynthesis and a mammalian GnTIII comprising crossing a plant comprising a functional protein such as a transporter or a (mammalian) enzyme or functional fragment thereof providing N-glycan biosynthesis with a plant according to Claim 5, harvesting progeny from said crossing and selecting a desired progeny plant expressing said functional protein such as a transporter or a (mammalian) enzyme or functional fragment thereof providing N-glycan biosynthesis and said mammalian GnTIII. In another embodiment, the present invention contemplates a transgenic plant obtained according to the

contemplated.

In one embodiment, the present invention contemplates a method for increasing galactosylation of a heterologous glycoprotein expressed in a plant host system comprising introducing a nucleic acid sequence encoding GnTIII and a sequence selected from a group consisting sequences that encode a transporter or a (mammalian) enzyme or functional fragment not normally present in a plant into said plant host system expressing said heterologous glycoprotein and isolating said glycoprotein.

In one embodiment, the present invention contemplates a plant derived glycoprotein comprising bisected oligosaccharides.

In one embodiment, the present invention contemplates the use of a plant host system contemplated by the present invention to produce a desired glycoprotein or functional fragment thereof. In another embodiment, the present invention contemplates that said glycoprotein or functional fragment thereof comprises bisected oligosaccharides. In yet another embodiment, the present invention contemplates a plant-derived glycoprotein or functional fragment thereof obtained by a method contemplated by the present invention. In yet another embodiment, the present invention contemplates a glycoprotein or functional fragment thereof contemplated by the invention for the production of a pharmaceutical composition. In yet another embodiment, the present invention contemplates a composition comprising a glycoprotein or functional fragment thereof as contemplated by the present invention.

In one embodiment, the present invention contemplates an isolated hybrid protein comprising an active site of GnTIII and a transmembrane region of a protein, said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell. In another embodiment, the present invention contemplates the protein of the present invention, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is an enzyme. In yet another embodiment, the present invention contemplates the protein accordof the present invention, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is a glycosyltransferase. In yet another embodiment, the present invention contemplates the protein of the present invention, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is a glycosyltransferase selected from the group consisting of a mannosidaseI, mannosidaseII, GnTI, GnTII, Xy1T and FucT. In yet another embodiment, the present invention contemplates the protein accorof the present invention, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is a plant protein. In yet another embodiment, the present invention contemplates an isolated nucleic acid sequence encoding the protein of the present invention. In yet another embodiment, the present invention contemplates a vector comprising the isolated nucleic acid sequence of the present invention. In yet another embodiment, the present invention contemplates a plant comprising the isolated nucleic acid sequence of the present invention. In yet another embodiment, the present invention contemplates the plant(s) of the present invention which further comprises a nucleic acid sequence encoding a heterologous glycoprotein.

In one embodiment, the present invention contemplates a method (for providing a transgenic

plant capable of expressing a heterologous glycoprotein with the capacity to extend an N-linked glycan with galactose) comprising a) crossing a transgenic plant with a plant of the present invention, b) harvesting progeny from said crossing and c) selecting a desired progeny plant (expressing said recombinant protein and expressing a functional (mammalian) enzyme involved in (mammalian) N-glycan biosynthesis that is normally not present in plants).

In one embodiment, the present invention contemplates a method for providing a transgenic plant capable of expressing a heterologous glycoprotein with the capacity to extend an N-linked glycan with galactose comprising introducing the nucleic acid sequence of the present invention and a nucleic acid sequence encoding said heterologous glycoprotein.

In one embodiment, the present invention contemplates a method, comprising: a) providing: i) a plant cell, and ii) an expression vector comprising nucleic acid encoding a GNTIII enzyme; and b) introducing said expression vector into said plant cell under conditions such that said enzyme is expressed. In another embodiment, the present invention contemplates the method, wherein said nucleic acid encoding a GNTIII comprises the nucleic acid sequence of SEQ ID NO:1.

In one embodiment, the present invention contemplates a method, comprising: a) providing: i) a plant cell, ii) a first expression vector comprising nucleic acid encoding a GNTIII enzyme, and iii) a second expression vector comprising nucleic acid encoding a heterologous glycoprotein; and b) introducing said first and second expression vectors into said plant cell under conditions such that said hybrid enzyme and said heterologous protein are expressed. In another embodiment, the present invention contemplates the method, wherein said heterologous protein is an antibody or antibody fragment.

In one embodiment, the present invention contemplates A method, comprising: a) providing: i) a first plant comprising a first expression vector, said first vector comprising nucleic acid encoding a GNTIII enzyme, and ii) a second plant comprising a second expression vector, said second vector comprising nucleic acid encoding a heterologous protein; and b) crossing said first plant and said second plant to produce progeny expressing said hybrid enzyme and said heterologous protein.

In one embodiment, the present invention contemplates a plant, comprising first and second expression vectors, said first vector comprising nucleic acid encoding a GNTIII enzyme, said second vector comprising nucleic acid encoding a heterologous protein. In another embodiment, the present invention contemplates the, wherein said heterologous protein is an antibody or antibody fragment.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B show MALDI-TOF mass spectra of (A) N-linked glycans isolated from leaves of control tobacco plant and (B) N-linked glycans isolated from leaves of selected GnTM-17 tobacco plant transformed with human GnTIII. See, Table 1 for structures.

Figure 2 shows processing of high mannose type glycan (M9) to complex type glycans under the subsequent action of ManI, GnTI, ManII, and GnTII. It is also indicated what glycan structures the action of GalT and/or GnTIII at different points

in chain of reactions would lead. The reactions catalyzed by fucosyltransferases and xylosyltransferases are not indicated. Core GlcNAc (Gn) is not indicated. Gn = GlcNAc, Gn^b = bisecting GlcNAc, G = galactose and M = mannose.

Figures 3A and 3B show (A) the T-DNA construct carrying the genes encoding glycan modifying enzymes to produce efficiently galactosylated bisected glycans that are devoid of immunogenic xylose and fucose and (B) the T-DNA construct carrying antibody light chain and heavy chain genes. TmXyl = transmembrane domain of xylosyltransferase, TmGnTI = transmembrane domain of GnT, P = promoter, R = selection marker, L = antibody light chain and H = antibody heavy chain.

Figures 4A and 4B show the nucleotide sequence (SEQ ID NO: 1, underlined portion of Figure 4(A) the protein sequence (SEQ ID NO: 2, underlined portion of Figure 4B) of *GnTIII* including a c-myc tag. Residues that can undergo conservative amino acid substitutions are defined in the DEFINITIONS section.

Figure 5A and 5B show a (A) map of the plasmid pDAB4005 and (B) the nucleotide sequence of the plasmid pDAB4005 (SEQ ID NO: 8).

Figure 6A and 6B show a (A) map of the plasmid pDAB7119 and (B) the nucleotide sequence of the plasmid pDAB7119 (SEQ ID NO: 9) including splice sites.

Figure 7A and 7B show a (A) map of the plasmid pDAB8504 and (B) the nucleotide sequence of the plasmid pDAB8504 (SEQ ID NO: 10).

Figure 8A and 8B show a (A) map of the plasmid pDAB7113 and (B) the nucleotide sequence of the plasmid pDAB7113 (SEQ ID NO: 11) including splice sites.

Figures 9A and 9B show MALDI-TOF mass spectra of glycoproteins from control and GnTIII corn. Comparison of mass spectra of N-glycans of glycoproteins isolated from calli of (A) control corn and of (B) selected GnTIII-corn. GnTIII corn was obtained through transformation with human GnTIII gene sequence and selection was performed by lectin blotting using E-PHA. See Table 3 for an annotation of the data contained in Figures 9A and 9B.

Figure 10 shows the full nucleotide sequence of *GntIII* without a c-myc tag (SEQ ID NO: 7).

Figure 11 shows a MALDI-TOF mass spectra of glycoproteins from control and GnTIII corn-2. See, Table 4 for structures and abbreviations.

Figure 12 shows a representative blot of samples of transgenic maize callus for altered lectin binding due to expression of the GntIII gene.

Figure 13 shows a representative blot of samples of transgenic maize callus for c-myc epitope expression.

Figures 14 A and 14B show a MALDI-TOF mass spectra of glycoproteins from (A) control and (B) GnTIII corn plants.

DEFINITIONS

The terms "protein" and "polypeptide" refer to compounds comprising amino acids joined via peptide bonds and are used interchangeably. A "protein" or "polypeptide" encoded by a gene is not

limited to the amino acid sequence encoded by the gene, but includes post-translational modifications of the protein.

The term “glycoprotein” refers to proteins with covalently attached sugar units, either bonded via the OH group of serine or threonine (O glycosylated) or through the amide NH2 of asparagine (N glycosylated). “Glycoprotein” may include, but is not limited to, for example, most secreted proteins (serum albumin is the major exception) and proteins exposed at the outer surface of the plasma membrane. Sugar residues found include, but are not limited to: mannose, N acetyl glucosamine, N acetyl galactosamine, galactose, fucose and sialic acid.

Where the term “amino acid sequence” is recited herein to refer to an amino acid sequence of a protein molecule, “amino acid sequence” and like terms, such as “polypeptide” or “protein” are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule. Furthermore, an “amino acid sequence” can be deduced from the nucleic acid sequence encoding the protein.

The term "portion" when used in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino sequence minus one amino acid.

The term "chimera" when used in reference to a polypeptide refers to the expression product of two or more coding sequences obtained from different genes, that have been cloned together and that, after translation, act as a single polypeptide sequence. Chimeric polypeptides are also referred to as "hybrid" polypeptides. The coding sequences includes those obtained from the same or from different species of organisms.

The term "fusion" when used in reference to a polypeptide refers to a chimeric protein containing a protein of interest joined to an exogenous protein fragment (the fusion partner). The fusion partner may serve various functions, including enhancement of solubility of the polypeptide of interest, as well as providing an "affinity tag" to allow purification of the recombinant fusion polypeptide from a host cell or from a supernatant or from both. If desired, the fusion partner may be removed from the protein of interest after or during purification.

The term "homolog" or "homologous" when used in reference to a polypeptide refers to a high degree of sequence identity between two polypeptides, or to a high degree of similarity between the three-dimensional structure or to a high degree of similarity between the active site and the mechanism of action. In a preferred embodiment, a homolog has a greater than 60 % sequence identity, and more preferably greater than 75% sequence identity, and still more preferably greater than 90 % sequence identity, with a reference sequence.

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (*e.g.*, 99 percent sequence identity). Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

The terms "variant" and "mutant" when used in reference to a polypeptide refer to an amino

acid sequence that differs by one or more amino acids from another, usually related polypeptide. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties. One type of conservative amino acid substitutions refers to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine (V) -leucine (L) -isoleucine (I), phenylalanine (F) -tyrosine (Y), lysine (K) -arginine (R), alanine (A) -valine (V), and asparagine (N) -glutamine (Q). More rarely, a variant may have "non-conservative" changes (*e.g.*, replacement of a glycine with a tryptophan). Similar minor variations may also include amino acid deletions or insertions (*i.e.*, additions), or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing biological activity may be found using computer programs well known in the art, for example, DNAStar software. Variants can be tested in functional assays. Preferred variants have less than 10 %, and preferably less than 5 %, and still more preferably less than 2 % changes (whether substitutions, deletions, and so on).

The term "domain" when used in reference to a polypeptide refers to a subsection of the polypeptide which possesses a unique structural and/or functional characteristic; typically, this characteristic is similar across diverse polypeptides. The subsection typically comprises contiguous amino acids, although it may also comprise amino acids which act in concert or which are in close proximity due to folding or other configurations.

The term "gene" refers to a nucleic acid (*e.g.*, DNA or RNA) sequence that comprises coding sequences necessary for the production of an RNA, or a polypeptide or its precursor (*e.g.*, proinsulin). A functional polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence as long as the desired activity or functional properties (*e.g.*, enzymatic activity, ligand binding, signal transduction, etc.) of the polypeptide are retained. The term "portion" when used in reference to a gene refers to fragments of that gene. The fragments may range in size from a few nucleotides (*e.g.*, ten nucleotides) to the entire gene sequence minus one nucleotide. Thus, "a nucleotide comprising at least a portion of a gene" may comprise fragments of the gene or the entire gene.

The term "gene" also encompasses the coding regions of a structural gene and includes sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb on either end such that the gene corresponds to the length of the full-length mRNA. The sequences which are located 5' of the coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' non-translated sequences. The term "gene"

encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene which are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

In addition to containing introns, genomic forms of a gene may also include sequences located on both the 5' and 3' end of the sequences which are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5' flanking region may contain regulatory sequences such as promoters and enhancers which control or influence the transcription of the gene. The 3' flanking region may contain sequences which direct the termination of transcription, posttranscriptional cleavage and polyadenylation.

The term "heterologous" when used in reference to a gene refers to a gene encoding a factor that is not in its natural environment (*i.e.*, has been altered by the hand of man). For example, a heterologous gene includes a gene from one species introduced into another species. A heterologous gene also includes a gene native to an organism that has been altered in some way (*e.g.*, mutated, added in multiple copies, linked to a non-native promoter or enhancer sequence, etc.). Heterologous genes may comprise gene sequences that comprise cDNA forms of a gene; the cDNA sequences may be expressed in either a sense (to produce mRNA) or anti-sense orientation (to produce an anti-sense RNA transcript that is complementary to the mRNA transcript). Heterologous genes are distinguished from endogenous genes in that the heterologous gene sequences are typically joined to nucleotide sequences comprising regulatory elements such as promoters that are not found naturally associated with the gene for the protein encoded by the heterologous gene or with gene sequences in the chromosome, or are associated with portions of the chromosome not found in nature (*e.g.*, genes expressed in loci where the gene is not normally expressed).

A "heterologous glycoprotein" is a glycoprotein originating from a species other than the plant host system. The glycoprotein may include but is not limited to antibodies, hormones, growth factors, and growth factor receptors, antigens, cytokines and blood products.

A "plant host system" may include, but is not limited to, a plant or portion thereof which includes, but is not limited to, a plant cell, plant organ and/or plant tissue. The plant may be a monocotyledon (monocot) which is a flowering plant whose embryos have one cotyledon or seed leaf and includes but is not limited to lilies, grasses, corn *(Zea mays)*, rice, grains including oats, wheat and barley, orchids, irises, onions and palms. Alternatively, the plant may be a dicotyledenon (dicot) which includes, but is not limited to, tobacco *(Nicotiana)*, tomatoes, potatoes, legumes (*e.g.*, alfalfa and soybeans), roses, daises, cacti, violets and duckweed. The plant may also be a moss which includes, but is not limited to, *Physcomitrella patens*. The invention is further directed to a method for obtaining said bisected GlcNAc in a plant host system by introducing a nucleic acid

encoding said GnTIII into a plant or portion thereof and expressing said GnTIII and isolating said plant or portion thereof expressing said GnTIII.

The term "nucleotide sequence of interest" or "nucleic acid sequence of interest" refers to any nucleotide sequence (*e.g.*, RNA or DNA), the manipulation of which may be deemed desirable for any reason (*e.g.*, treat disease, confer improved qualities, *etc.*), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (*e.g.*, reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, *etc.*), and non-coding regulatory sequences which do not encode an mRNA or protein product (*e.g.*, promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence and other like sequences). The present invention contemplates host cells expressing a heterologous protein encoded by a nucleotide sequence of interest along with one or more hybrid enzymes.

The term "structural" when used in reference to a gene or to a nucleotide or nucleic acid sequence refers to a gene or a nucleotide or nucleic acid sequence whose ultimate expression product is a protein (such as an enzyme or a structural protein), an rRNA, an sRNA, a tRNA, etc.

The terms "oligonucleotide" or "polynucleotide" or "nucleotide" or "nucleic acid" refer to a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three, and usually more than ten. The exact size will depend on many factors, which in turn depends on the ultimate function or use of the oligonucleotide. The oligonucleotide may be generated in any manner, including chemical synthesis, DNA replication, reverse transcription, or a combination thereof.

The terms "an oligonucleotide having a nucleotide sequence encoding a gene" or "a nucleic acid sequence encoding" a specified polypeptide refer to a nucleic acid sequence comprising the coding region of a gene or in other words the nucleic acid sequence which encodes a gene product. The coding region may be present in either a cDNA, genomic DNA or RNA form. When present in a DNA form, the oligonucleotide may be single-stranded (*i.e.*, the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, *etc.* may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, *etc.* or a combination of both endogenous and exogenous control elements.

The term "recombinant" when made in reference to a nucleic acid molecule refers to a nucleic acid molecule which is comprised of segments of nucleic acid joined together by means of molecular biological techniques. The term "recombinant" when made in reference to a protein or a polypeptide refers to a protein molecule which is expressed using a recombinant nucleic acid molecule.

As used herein, the terms "complementary" or "complementarity" are used in reference to nucleotide sequences related by the base-pairing rules. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial"

complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.

A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acids show total complementarity to the nucleic acids of the nucleic acid sequence. For example, the present invention contemplates the complements of SEQ ID NO: 1.

The term "homology" when used in relation to nucleic acids refers to a degree of complementarity. There may be partial homology (*i.e.*, partial identity) or complete homology (*i.e.*, complete identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid and is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe (*i.e.*, an oligonucleotide which is capable of hybridizing to another oligonucleotide of interest) will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous sequence to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target which lacks even a partial degree of complementarity (*e.g.*, less than about 30 % identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe which can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described *infra*.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe which can hybridize to the single-stranded nucleic acid sequence under conditions of low stringency as described *infra*.

The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence," "sequence identity," "percentage of sequence identity" and "substantial identity." A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA sequence given in a sequence listing or may comprise a complete gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (*i.e.*, a portion of the complete polynucleotide sequence) that is similar between

the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window," as used herein, refers to a conceptual segment of at least 20 contiguous nucleotide positions wherein a polynucleotide sequence may be compared to a reference sequence of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman [Smith and Waterman, *Adv. Appl. Math.* 2: 482 (1981)] by the homology alignment algorithm of Needleman and Wunsch [Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970)], by the search for similarity method of Pearson and Lipman [Pearson and Lipman, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988)], by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (*i.e.*, resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected. The term "sequence identity" means that two polynucleotide sequences are identical (*i.e.*, on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 25-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison. The reference sequence may be a subset of a larger sequence, for example, as a segment of the full-length sequences of the compositions claimed in the present invention.

The term "hybridization" refers to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. A single molecule that contains pairing of complementary nucleic acids within its structure is said to be

"self-hybridized."

The term "T_m" refers to the "melting temperature" of a nucleic acid. The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (*See e.g.*, Anderson and Young, Quantitative Filter Hybridization, *in Nucleic Acid Hybridization* [1985]). Other references include more sophisticated computations that take structural as well as sequence characteristics into account for the calculation of T_m.

The term "stringency" refers to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences. Thus, conditions of "low" stringency are often required with nucleic acids that are derived from organisms that are genetically diverse, as the frequency of complementary sequences is usually less.

"Low stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42 ^{O}C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l $NaH_2PO_4(H_2O$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42 ^{O}C when a probe of about 500 nucleotides in length is employed.

"Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42 ^{O}C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l $NaH_2PO_4(H_2O$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5 % SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0 % SDS at 42 ^{O}C when a probe of about 500 nucleotides in length is employed.

"High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42 ^{O}C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l $NaH_2PO_4(H_2O$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5 % SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0 % SDS at 42 ^{O}C when a probe of about 500 nucleotides in length is employed.

It is well known that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may

be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.).

Additionally, the term "equivalent," when made in reference to a hybridization condition as it relates to a hybridization condition of interest, means that the hybridization condition and the hybridization condition of interest result in hybridization of nucleic acid sequences which have the same range of percent (%) homology. For example, if a hybridization condition of interest results in hybridization of a first nucleic acid sequence with other nucleic acid sequences that have from 50 % to 70 % homology to the first nucleic acid sequence, then another hybridization condition is said to be equivalent to the hybridization condition of interest if this other hybridization condition also results in hybridization of the first nucleic acid sequence with the other nucleic acid sequences that have from 50 % to 70 % homology to the first nucleic acid sequence.

When used in reference to nucleic acid hybridization the art knows well that numerous equivalent conditions may be employed to comprise either low or high stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency hybridization different from, but equivalent to, the above-listed conditions.

The term "wild-type" when made in reference to a gene refers to a gene that has the characteristics of a gene isolated from a naturally occurring source. The term "wild-type" when made in reference to a gene product refers to a gene product that has the characteristics of a gene product isolated from a naturally occurring source. The term "naturally-occurring" as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring. A wild-type gene is frequently that gene which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" when made in reference to a gene or to a gene product refers, respectively, to a gene or to a gene product which displays modifications in sequence and/or functional properties (*i.e.*, altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

Thus, the terms "variant" and "mutant" when used in reference to a nucleotide sequence refer to an nucleic acid sequence that differs by one or more nucleotides from another, usually related nucleotide acid sequence. A "variation" is a difference between two different nucleotide sequences; typically, one sequence is a reference sequence.

The term "polymorphic locus" refers to a genetic locus present in a population that shows

variation between members of the population (*i.e.*, the most common allele has a frequency of less than 0.95). Thus, "polymorphism" refers to the existence of a character in two or more variant forms in a population. A "single nucleotide polymorphism" (or SNP) refers a genetic locus of a single base which may be occupied by one of at least two different nucleotides. In contrast, a "monomorphic locus" refers to a genetic locus at which little or no variations are seen between members of the population (generally taken to be a locus at which the most common allele exceeds a frequency of 0.95 in the gene pool of the population).

A "frameshift mutation" refers to a mutation in a nucleotide sequence, usually resulting from insertion or deletion of a single nucleotide (or two or four nucleotides) which results in a change in the correct reading frame of a structural DNA sequence encoding a protein. The altered reading frame usually results in the translated amino-acid sequence being changed or truncated.

A "splice mutation" refers to any mutation that affects gene expression by affecting correct RNA splicing. Splicing mutation may be due to mutations at intron-exon boundaries which alter splice sites.

The term "detection assay" refers to an assay for detecting the presence or absence of a sequence or a variant nucleic acid sequence (*e.g.*, mutation or polymorphism in a given allele of a particular gene, as *e.g.*, *GnTIII* gene, SEQ ID NO: 1, Figure 4A), or for detecting the presence or absence of a particular protein (*e.g.*, GnTIII, SEQ ID NO: 2, Figure 4B) or the structure or activity or effect of a particular protein (*e.g.*, GnTIII activity), for detecting glycosylation moieties on a particular protein (*e.g.*, N-linked glycans) or for detecting the presence or absence of a variant of a particular protein.

The term "antisense" refers to a deoxyribonucleotide sequence whose sequence of deoxyribonucleotide residues is in reverse 5' to 3' orientation in relation to the sequence of deoxyribonucleotide residues in a sense strand of a DNA duplex. A "sense strand" of a DNA duplex refers to a strand in a DNA duplex which is transcribed by a cell in its natural state into a "sense mRNA." Thus an "antisense" sequence is a sequence having the same sequence as the non-coding strand in a DNA duplex. The term "antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene by interfering with the processing, transport and/or translation of its primary transcript or mRNA. The complementarity of an antisense RNA may be with any part of the specific gene transcript, *i.e.*, at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. In addition, as used herein, antisense RNA may contain regions of ribozyme sequences that increase the efficacy of antisense RNA to block gene expression. "Ribozyme" refers to a catalytic RNA and includes sequence-specific endoribonucleases. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of preventing the expression of the target protein.

"Amplification" is a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (*i.e.*, replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of

replication (*i.e.*, synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out.

Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Q∃ replicase, MDV-1 RNA is the specific template for the replicase (Kacian *et al.*, Proc. Natl. Acad. Sci. USA, 69:3038, 1972). Other nucleic acid will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (Chamberlain *et al.*, Nature, 228:227, 1970). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (Wu and Wallace, Genomics, 4:560, 1989). Finally, *Taq* and *Pfu* polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences (H.A. Erlich (ed.), *PCR Technology*, Stockton Press, 1989).

The term "amplifiable nucleic acid" refers to nucleic acids that may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" will usually comprise "sample template."

The term "sample template" refers to nucleic acid originating from a sample that is analyzed for the presence of "target" (defined below). In contrast, "background template" is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is most often inadvertent. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

The term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, (*i.e.*, in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

The term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether

occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, that is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labeled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

The term "target," when used in reference to the polymerase chain reaction, refers to the region of nucleic acid bounded by the primers used for polymerase chain reaction. Thus, the "target" is sought to be sorted out from other nucleic acid sequences. A "segment" is defined as a region of nucleic acid within the target sequence.

The term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, that describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified."

With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (*e.g.*, hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

The terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant

mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

The term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

The term "reverse-transcriptase" or "RT-PCR" refers to a type of PCR where the starting material is mRNA. The starting mRNA is enzymatically converted to complementary DNA or "cDNA" using a reverse transcriptase enzyme. The cDNA is then used as a "template" for a "PCR" reaction

The term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (*e.g.*, mRNA, rRNA, tRNA, or snRNA) through "transcription" of the gene (*i.e.*, via the enzymatic action of an RNA polymerase), and into protein, through "translation" of mRNA. Gene expression can be regulated at many stages in the process. "Up-regulation" or "activation" refers to regulation that increases the production of gene expression products (*i.e.*, RNA or protein), while "down-regulation" or "repression" refers to regulation that decrease production. Molecules (*e.g.*, transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors," respectively.

The terms "in operable combination," "in operable order" and "operably linked" refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

The term "regulatory element" refers to a genetic element which controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, *etc.*

Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis, *et al.*, Science 236:1237, 1987). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect, mammalian and plant cells. Promoter and enhancer elements have also been isolated from viruses and analogous control elements, such as promoters, are also found in prokaryotes. The selection of a particular promoter and enhancer depends on the cell type used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types (*for review, see* Voss, *et al.*, Trends Biochem. Sci., 11:287, 1986; and Maniatis, *et al.*, *supra* 1987).

The terms "promoter element," "promoter" or "promoter sequence" refer to a DNA sequence

that is located at the 5' end (*i.e.* precedes) of the coding region of a DNA polymer. The location of most promoters known in nature precedes the transcribed region. The promoter functions as a switch, activating the expression of a gene. If the gene is activated, it is said to be transcribed, or participating in transcription. Transcription involves the synthesis of mRNA from the gene. The promoter, therefore, serves as a transcriptional regulatory element and also provides a site for initiation of transcription of the gene into mRNA.

Promoters may be tissue specific or cell specific. The term "tissue specific" as it applies to a promoter refers to a promoter that is capable of directing selective expression of a nucleotide sequence of interest to a specific type of tissue (*e.g.*, petals) in the relative absence of expression of the same nucleotide sequence of interest in a different type of tissue (*e.g.*, roots). Tissue specificity of a promoter may be evaluated by, for example, operably linking a reporter gene to the promoter sequence to generate a reporter construct, introducing the reporter construct into the genome of a plant such that the reporter construct is integrated into every tissue of the resulting transgenic plant, and detecting the expression of the reporter gene (*e.g.*, detecting mRNA, protein, or the activity of a protein encoded by the reporter gene) in different tissues of the transgenic plant. The detection of a greater level of expression of the reporter gene in one or more tissues relative to the level of expression of the reporter gene in other tissues shows that the promoter is specific for the tissues in which greater levels of expression are detected. The term "cell type specific" as applied to a promoter refers to a promoter which is capable of directing selective expression of a nucleotide sequence of interest in a specific type of cell in the relative absence of expression of the same nucleotide sequence of interest in a different type of cell within the same tissue. The term "cell type specific" when applied to a promoter also means a promoter capable of promoting selective expression of a nucleotide sequence of interest in a region within a single tissue. Cell type specificity of a promoter may be assessed using methods well known in the art, *e.g.*, immunohistochemical staining. Briefly, tissue sections are embedded in paraffin, and paraffin sections are reacted with a primary antibody which is specific for the polypeptide product encoded by the nucleotide sequence of interest whose expression is controlled by the promoter. A labeled (*e.g.*, peroxidase conjugated) secondary antibody which is specific for the primary antibody is allowed to bind to the sectioned tissue and specific binding detected (*e.g.*, with avidin/biotin) by microscopy.

Promoters may be constitutive or regulatable. The term "constitutive" when made in reference to a promoter means that the promoter is capable of directing transcription of an operably linked nucleic acid sequence in the absence of a stimulus (*e.g.*, heat shock, chemicals, light, *etc.*). Typically, constitutive promoters are capable of directing expression of a transgene in substantially any cell and any tissue. In contrast, a "regulatable" promoter is one which is capable of directing a level of transcription of an operably linked nuclei acid sequence in the presence of a stimulus (*e.g.*, heat shock, chemicals, light, *etc.*) which is different from the level of transcription of the operably linked nucleic acid sequence in the absence of the stimulus.

The terms "infecting" and "infection" with a bacterium refer to co-incubation of a target biological sample, (*e.g.*, cell, tissue, *etc.*) with the bacterium under conditions such that nucleic acid

sequences contained within the bacterium are introduced into one or more cells of the target biological sample.

The term "*Agrobacterium*" refers to a soil-borne, Gram-negative, rod-shaped phytopathogenic bacterium which causes crown gall. The term "*Agrobacterium*" includes, but is not limited to, the strains *Agrobacterium tumefaciens*, (which typically causes crown gall in infected plants), and *Agrobacterium rhizogens* (which causes hairy root disease in infected host plants). Infection of a plant cell with *Agrobacterium* generally results in the production of opines (e.g., nopaline, agropine, octopine etc.) by the infected cell. Thus, *Agrobacterium* strains which cause production of nopaline (*e.g.*, strain LBA4301, C58, A208) are referred to as "nopaline-type" *Agrobacteria*; *Agrobacterium* strains which cause production of octopine (*e.g.*, strain LBA4404, Ach5, B6) are referred to as "octopine-type" *Agrobacteria*; and *Agrobacterium* strains which cause production of agropine (*e.g.*, strain EHA105, EHA101, A281) are referred to as "agropine-type" *Agrobacteria*.

The term "regulatory region" refers to a gene's 5' transcribed but untranslated regions, located immediately downstream from the promoter and ending just prior to the translational start of the gene.

The term "promoter region" refers to the region immediately upstream of the coding region of a DNA polymer, and is typically between about 500 bp and 4 kb in length, and is preferably about 1 to 1.5 kb in length.

In contrast, an "inducible" promoter is one which is capable of directing a level of transcription of an operably linked nucleic acid sequence in the presence of a stimulus (*e.g.*, heat shock, chemicals, light, *etc.*) which is different from the level of transcription of the operably linked nucleic acid sequence in the absence of the stimulus.

The term "regulatory element" refers to a genetic element that controls some aspect of the expression of nucleic acid sequence(s). For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, etc.

The enhancer and/or promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer or promoter is one that is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer or promoter is one that is placed in juxtaposition to a gene by means of genetic manipulation (*i.e.*, molecular biological techniques) such that transcription of the gene is directed by the linked enhancer or promoter. For example, an endogenous promoter in operable combination with a first gene can be isolated, removed, and placed in operable combination with a second gene, thereby making it a "heterologous promoter" in operable combination with the second gene. A variety of such combinations are contemplated (*e.g.*, the first and second genes can be from the same species, or from different species).

The term "naturally linked" or "naturally located" when used in reference to the relative positions of nucleic acid sequences means that the nucleic acid sequences exist in nature in the relative positions.

The presence of "splicing signals" on an expression vector often results in higher levels of expression of the recombinant transcript in eukaryotic host cells. Splicing signals mediate the removal of introns from the primary RNA transcript and consist of a splice donor and acceptor site (Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, New York [1989] pp. 16.7-16.8). A commonly used splice donor and acceptor site is the splice junction from the 16S RNA of SV40.

Efficient expression of recombinant DNA sequences in eukaryotic cells requires expression of signals directing the efficient termination and polyadenylation of the resulting transcript. Transcription termination signals are generally found downstream of the polyadenylation signal and are a few hundred nucleotides in length. The term "poly(A) site" or "poly(A) sequence" as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript. Efficient polyadenylation of the recombinant transcript is desirable, as transcripts lacking a poly(A) tail are unstable and are rapidly degraded. The poly(A) signal utilized in an expression vector may be "heterologous" or "endogenous." An endogenous poly(A) signal is one that is found naturally at the 3' end of the coding region of a given gene in the genome. A heterologous poly(A) signal is one which has been isolated from one gene and positioned 3' to another gene. A commonly used heterologous poly(A) signal is the SV40 poly(A) signal. The SV40 poly(A) signal is contained on a 237 bp *Bam*HI/*Bcl*I restriction fragment and directs both termination and polyadenylation (Sambrook, *supra*, at 16.6-16.7).

The term "vector" refers to any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, retrovirus, virion, or similar genetic element, which is capable of replication when associated with the proper control elements and which can transfer gene sequences into cells and/or between cells. Thus, this term includes cloning and expression vehicles, as well as viral vectors.

The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence (or coding sequences) - such as the coding sequence(s) for the hybrid enzyme(s) described in more detail below - and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host cell or organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a ribosome binding site, often along with other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. It is not intended that the present invention be limited to particular expression vectors or expression vectors with particular elements.

The term "transfection" refers to the introduction of foreign DNA into cells. Transfection may be accomplished by a variety of means known to the art including calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection, polybrene-mediated transfection, glass beads, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, viral infection, biolistics (*i.e.*, particle bombardment) and the like.

The term "stable transfection" or "stably transfected" refers to the introduction and

integration of foreign DNA into the genome of the transfected cell. The term "stable transfectant" refers to a cell that has stably integrated foreign DNA into the genomic DNA.

The term "transient transfection" or "transiently transfected" refers to the introduction of foreign DNA into a cell where the foreign DNA fails to integrate into the genome of the transfected cell. The foreign DNA persists in the nucleus of the transfected cell for several days. During this time the foreign DNA is subject to the regulatory controls that govern the expression of endogenous genes in the chromosomes. The term "transient transfectant" refers to cells that have taken up foreign DNA but have failed to integrate this DNA.

The term "calcium phosphate co-precipitation" refers to a technique for the introduction of nucleic acids into a cell. The uptake of nucleic acids by cells is enhanced when the nucleic acid is presented as a calcium phosphate-nucleic acid co-precipitate. The original technique of Graham and van der Eb (Graham and van der Eb, *Virol.*, 52:456, 1973), has been modified by several groups to optimize conditions for particular types of cells. The art is well aware of these numerous modifications.

The terms "bombarding, "bombardment," and "biolistic bombardment" refer to the process of accelerating particles towards a target biological sample (*e.g.*, cell, tissue, *etc.*) to effect wounding of the cell membrane of a cell in the target biological sample and/or entry of the particles into the target biological sample. Methods for biolistic bombardment are known in the art (*e.g.*, U.S. Patent No. 5,584,807, the contents of which are incorporated herein by reference), and are commercially available (*e.g.*, the helium gas-driven microprojectile accelerator (PDS-1000/He, BioRad).

The term "microwounding" when made in reference to plant tissue refers to the introduction of microscopic wounds in that tissue. Microwounding may be achieved by, for example, particle bombardment as described herein.

The term "plant" as used herein refers to a plurality of plant cells which are largely differentiated into a structure that is present at any stage of a plant's development. Such structures include, but are not limited to, a fruit, shoot, stem, leaf, flower petal, *etc.* The term "plant tissue" includes differentiated and undifferentiated tissues of plants including, but not limited to, roots, shoots, leaves, pollen, seeds, tumor tissue and various types of cells in culture (*e.g.*, single cells, protoplasts, embryos, callus, protocorm-like bodies, *etc.*). Plant tissue may be *in planta*, in organ culture, tissue culture, or cell culture. Similarly, "plant cell(s)" may be cells in culsture or may be part of a plant.

The term "transgenic" when used in reference to a cell refers to a cell which contains a transgene, or whose genome has been altered by the introduction of a transgene. The term "transgenic" when used in reference to a cell, tissue or to a plant refers to a cell, tissue or plant, respectively, which comprises a transgene, where one or more cells of the tissue contain a transgene (such as a gene encoding the hybrid enzyme(s) of the present invention), or a plant whose genome has been altered by the introduction of a transgene. Transgenic cells, tissues and plants may be produced by several methods including the introduction of a "transgene" comprising nucleic acid (usually DNA) into a target cell or integration of the transgene into a chromosome of a target cell by

way of human intervention, such as by the methods described herein.

The term "transgene" as used herein refers to any nucleic acid sequence which is introduced into the genome of a cell by experimental manipulations. A transgene may be an "endogenous DNA sequence," or a "heterologous DNA sequence" (i.e., "foreign DNA"). The term "endogenous DNA sequence" refers to a nucleotide sequence which is naturally found in the cell into which it is introduced so long as it does not contain some modification (e.g., a point mutation, the presence of a selectable marker gene, or other like modifications) relative to the naturally-occurring sequence. The term "heterologous DNA sequence" refers to a nucleotide sequence which is ligated to, or is manipulated to become ligated to, a nucleic acid sequence to which it is not ligated in nature, or to which it is ligated at a different location in nature. Heterologous DNA is not endogenous to the cell into which it is introduced, but has been obtained from another cell. Heterologous DNA also includes an endogenous DNA sequence which contains some modification. Generally, although not necessarily, heterologous DNA encodes RNA and proteins that are not normally produced by the cell into which it is expressed. Examples of heterologous DNA include reporter genes, transcriptional and translational regulatory sequences, selectable marker proteins (e.g., proteins which confer drug resistance), or other similar elements.

The term "foreign gene" refers to any nucleic acid (e.g., gene sequence) which is introduced into the genome of a cell by experimental manipulations and may include gene sequences found in that cell so long as the introduced gene contains some modification (e.g., a point mutation, the presence of a selectable marker gene, or other like modifications) relative to the naturally-occurring gene.

The term "transformation" as used herein refers to the introduction of a transgene into a cell. Transformation of a cell may be stable or transient. The term "transient transformation" or "transiently transformed" refers to the introduction of one or more transgenes into a cell in the absence of integration of the transgene into the host cell's genome. Transient transformation may be detected by, for example, enzyme-linked immunosorbent assay (ELISA) which detects the presence of a polypeptide encoded by one or more of the transgenes. Alternatively, transient transformation may be detected by detecting the activity of the protein (*e.g.*, β-glucuronidase) encoded by the transgene (*e.g.*, the *uid A* gene) as demonstrated herein (*e.g.*, histochemical assay of GUS enzyme activity by staining with X-gluc which gives a blue precipitate in the presence of the GUS enzyme; and a chemiluminescent assay of GUS enzyme activity using the GUS-Light kit (Tropix)). The term "transient transformant" refers to a cell which has transiently incorporated one or more transgenes. In contrast, the term "stable transformation" or "stably transformed" refers to the introduction and integration of one or more transgenes into the genome of a cell. Stable transformation of a cell may be detected by Southern blot hybridization of genomic DNA of the cell with nucleic acid sequences which are capable of binding to one or more of the transgenes. Alternatively, stable transformation of a cell may also be detected by the polymerase chain reaction of genomic DNA of the cell to amplify transgene sequences. The term "stable transformant" refers to a cell which has stably integrated one or more transgenes into the genomic DNA. Thus, a stable transformant is

distinguished from a transient transformant in that, whereas genomic DNA from the stable transformant contains one or more transgenes, genomic DNA from the transient transformant does not contain a transgene.

The term “host cell” refers to any cell capable of replicating and/or transcribing and/or translating a heterologous gene. Thus, a "host cell" refers to any eukaryotic or prokaryotic cell (*e.g.*, bacterial cells such as *E. coli*, yeast cells, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located *in vitro* or *in vivo*. For example, host cells may be located in a transgenic animal.

The terms "transformants" or "transformed cells" include the primary transformed cell and cultures derived from that cell without regard to the number of transfers. All progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same functionality as screened for in the originally transformed cell are included in the definition of transformants.

The term "selectable marker" refers to a gene which encodes an enzyme having an activity that confers resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed, or which confers expression of a trait which can be detected (*e.g.*., luminescence or fluorescence). Selectable markers may be "positive" or "negative." Examples of positive selectable markers include the neomycin phosphotrasferase (NPTII) gene which confers resistance to G418 and to kanamycin, and the bacterial hygromycin phosphotransferase gene (*hyg*), which confers resistance to the antibiotic hygromycin. Negative selectable markers encode an enzymatic activity whose expression is cytotoxic to the cell when grown in an appropriate selective medium. For example, the HSV-*tk* gene is commonly used as a negative selectable marker. Expression of the HSV-*tk* gene in cells grown in the presence of gancyclovir or acyclovir is cytotoxic; thus, growth of cells in selective medium containing gancyclovir or acyclovir selects against cells capable of expressing a functional HSV TK enzyme.

The term “reporter gene” refers to a gene encoding a protein that may be assayed. Examples of reporter genes include, but are not limited to, luciferase (*See, e.g.*, deWet *et al.*, *Mol. Cell. Biol.* 7:725, 1987 and U.S. Pat Nos.,6,074,859; 5,976,796; 5,674,713; and 5,618,682; all of which are incorporated herein by reference), green fluorescent protein (*e.g.*, GenBank Accession Number U43284; a number of GFP variants are commercially available from CLONTECH Laboratories, Palo Alto, CA), chloramphenicol acetyltransferase, ß-galactosidase, alkaline phosphatase, and horse radish peroxidase.

The term "overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. The term "cosuppression" refers to the expression of a foreign gene which has substantial homology to an endogenous gene resulting in the suppression of expression of both the foreign and the endogenous gene. As used herein, the term "altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

The terms "Southern blot analysis" and "Southern blot" and "Southern" refer to the analysis of DNA on agarose or acrylamide gels in which DNA is separated or fragmented according to size followed by transfer of the DNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized DNA is then exposed to a labeled probe to detect DNA species complementary to the probe used. The DNA may be cleaved with restriction enzymes prior to electrophoresis. Following electrophoresis, the DNA may be partially depurinated and denatured prior to or during transfer to the solid support. Southern blots are a standard tool of molecular biologists (J. Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, NY, pp 9.31-9.58, 1989).

The term "Northern blot analysis" and "Northern blot" and "Northern" refer to the analysis of RNA by electrophoresis of RNA on agarose gels to fractionate the RNA according to size followed by transfer of the RNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized RNA is then probed with a labeled probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists (J. Sambrook, *et al.*, *supra*, pp 7.39-7.52, 1989).

The terms "Western blot analysis" and "Western blot" and "Western" refers to the analysis of protein(s) (or polypeptides) immobilized onto a support such as nitrocellulose or a membrane. A mixture comprising at least one protein is first separated on an acrylamide gel, and the separated proteins are then transferred from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized proteins are exposed to at least one antibody with reactivity against at least one antigen of interest. The bound antibodies may be detected by various methods, including the use of radiolabeled antibodies.

The term "antigenic determinant" refers to that portion of an antigen that makes contact with a particular antibody (*i.e.*, an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies that bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (*i.e.*, the "immunogen" used to elicit the immune response) for binding to an antibody.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated nucleic acid sequence" refers to a nucleic acid sequence that is identified and separated from one or more other components (*e.g.*, separated from a cell containing the nucleic acid, or separated from at least one contaminant nucleic acid, or separated from one or more proteins, one or more lipids) with which it is ordinarily associated in its natural source. Isolated nucleic acid is nucleic acid present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids are nucleic acids such as DNA and RNA which are found in the state they exist in nature. For example, a given DNA sequence (*e.g.*, a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs which encode a multitude of proteins. However, an isolated nucleic acid sequence comprising, for example, SEQ ID NO:1 includes, by way

of example, such nucleic acid sequences in cells which ordinarily contain, for example, SEQ ID NO:1 where the nucleic acid sequence is in a chromosomal or extrachromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid sequence may be present in single-stranded or double-stranded form. When an isolated nucleic acid sequence is to be utilized to express a protein, the nucleic acid sequence will contain at a minimum at least a portion of the sense or coding strand (*i.e.*, the nucleic acid sequence may be single-stranded). Alternatively, it may contain both the sense and anti-sense strands (*i.e.*, the nucleic acid sequence may be double-stranded).

The term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment (or components of their natural environment), isolated or separated. An "isolated nucleic acid sequence" may therefore be a purified nucleic acid sequence. "Substantially purified" molecules are at least 60 % free, preferably at least 75 % free, and more preferably at least 90 % free from other components with which they are naturally associated. As used herein, the term "purified" or "to purify" also refer to the removal of contaminants from a sample. The removal of contaminating proteins results in an increase in the percent of polypeptide of interest in the sample. In another example, recombinant polypeptides are expressed in plant, bacterial, yeast, or mammalian host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant polypeptides is thereby increased in the sample. The present invention contemplates both purified (including substantially purified) and unpurified hybrid enzyme(s).

The term "composition comprising" a given polynucleotide sequence or polypeptide refers broadly to any composition containing the given polynucleotide sequence or polypeptide. The composition may comprise an aqueous solution. Compositions comprising polynucleotide sequences encoding GnTIII or fragments thereof may be employed as hybridization probes. In this case, the GnTIII encoding polynucleotide sequences are typically employed in an aqueous solution containing salts (*e.g.*, NaCl), detergents (*e.g.*, SDS), and other components (*e.g.*, Denhardt's solution, dry milk, salmon sperm DNA, etc.).

The term "test compound" refers to any chemical entity, pharmaceutical, drug, and the like that can be used to treat or prevent a disease, illness, sickness, or disorder of bodily function, or otherwise alter the physiological or cellular status of a sample. Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by screening using the screening methods of the present invention. A "known therapeutic compound" refers to a therapeutic compound that has been shown (*e.g.*, through animal trials or prior experience with administration to humans) to be effective in such treatment or prevention.

As used herein, the term "response," when used in reference to an assay, refers to the generation of a detectable signal (*e.g.*, accumulation of reporter protein, increase in ion concentration, accumulation of a detectable chemical product).

The term "sample" is used in its broadest sense. In one sense it can refer to a animal cell or tissue. In another sense, it is meant to include a specimen or culture obtained from any source, as

well as biological and environmental samples. Biological samples may be obtained from plants or animals (including humans) and encompass fluids, solids, tissues, and gases. Environmental samples include environmental material such as surface matter, soil, water, and industrial samples. These examples are not to be construed as limiting the sample types applicable to the present invention.

The term "fusion protein" refers to a protein wherein at least one part or portion is from a first protein and another part or portion is from a second protein. The term "hybrid enzyme" refers to a fusion protein which is a functional enzyme, wherein at least one part or portion is from a first species and another part or portion is from a second species. Preferred hybrid enzymes of the present invention are functional glycosyltransferases (or portions thereof) wherein at least one part or portion is from a plant and another part or portion is from a mammal (such as human).

The term "introduction into a cell" in the context of nucleic acid (*e.g.,* vectors) is intended to include what the art calls "transformation" or "transfection" or "transduction." Transformation of a cell may be stable or transient – and the present invention contemplates introduction of vectors under conditions where, on the one hand, there is stable expression, and on the other hand, where there is only transient expression. The term "transient transformation" or "transiently transformed" refers to the introduction of one or more transgenes into a cell in the absence of integration of the transgene into the host cell's genome. Transient transformation may be detected by, for example, enzyme-linked immunosorbent assay (ELISA) which detects the presence of a polypeptide encoded by one or more of the transgenes. Alternatively, transient transformation may be detected by detecting the activity of the protein (*e.g.,* antigen binding of an antibody) encoded by the transgene (*e.g.,* the antibody gene). The term "transient transformant" refers to a cell which has transiently incorporated one or more transgenes. In contrast, the term "stable transformation" or "stably transformed" refers to the introduction and integration of one or more transgenes into the genome of a cell. Stable transformation of a cell may be detected by Southern blot hybridization of genomic DNA of the cell with nucleic acid sequences which are capable of binding to one or more of the transgenes. Alternatively, stable transformation of a cell may also be detected by the polymerase chain reaction (PCR) of genomic DNA of the cell to amplify transgene sequences. The term "stable transformant" refers to a cell which has stably integrated one or more transgenes into the genomic DNA. Thus, a stable transformant is distinguished from a transient transformant in that, whereas genomic DNA from the stable transformant contains one or more transgenes, genomic DNA from the transient transformant does not contain a transgene.

"Bisected oligosaccharide" shall be defined as an oligosaccharide comprising, *e.g.*, two mannose groups and another saccharide moitey attached to a mannose residue of the oligosaccharide. Examples of bisected oligonucleotides are given in Table 1.

DETAILED DESCRIPTION OF THE INVENTION

The GnTIII (for example, SEQ ID NO: 1, Figure 4A) expressed in the plant host cell of the present invention is a mammalian GnTIII. In a specific embodiment, the GnTIII is a human GnTIII (for example, SEQ ID NO: 2, Figure 4B). The GnTIII may also in a specific embodiment have 80 %

identity with the amino acid sequence of a mammalian GnTIII, more preferably at least about 90 %, even more preferably at least about 95 %, and most preferably at least about 97 % (hereinafter "homologous polypeptides"), which qualitative retain the activity of said mammalian GnTIII. A polypeptide that has an amino acid sequence at least, for example, 95 % "identical" to a query amino acid sequence is identical to the query sequence except that the subject polypeptide sequence may include on average, up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95 % identical to a query amino acid sequence, up to 5 % of the amino acid residues in the subject sequence may be inserted, deleted or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the referenced sequence or in one or more contiguous groups within the reference sequence.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag, *et al.* (*Com. App. Biosci.* 6:237-245, 1990). In a sequence alignment, the query and subject sequence are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score= 1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C- terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C- terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

The GnTIII expressed in the plant host system of the present invention is encoded by a nucleic acid sequence that has at least 80 % identity with the nucleic acid sequence encoding an

amino acid sequence of a mammalian GnTIII, more preferably at least about 90 %, even more preferably at least about 95 %, and most preferably at least about 97 % (hereinafter "homologous polypeptides"), which qualitative retain the activity of said mammalian GnTIII. The nucleic acid sequence may be an RNA or DNA sequence.

A polynucleotide having 95 % "identity" to a reference nucleotide sequence of the present invention, is identical to the reference sequence except that the polynucleotide sequence may include, on average, up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95 % identical to a reference nucleotide sequence, up to 5 % of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5 % of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 90 %, 95 %, 96 %, 97 %, 98 % or 99 % identical to a nucleotide sequence of the presence invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag, *et al.*, (*Comp. App. Biosci.*, 6:237-245, 1990). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's (uridine) to T's (thymines). The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

The invention also encompasses polynucleotides that hybridize to the nucleic acid sequence encoding said mammalian GnTIII. A polynucleotide "hybridizes" to another polynucleotide, when a single-stranded form of the polynucleotide can anneal to the other polynucleotide under the appropriate conditions of temperature and solution ionic strength (see, Sambrook, *et al.*, supra). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a temperature of 42 ^{O}C, can be used, *e.g.*, 5X SSC, 0.1 % SDS, 0.25 % milk, and no formamide; or 40 % formamide, 5X SSC, 0.5 % SDS). Moderate stringency hybridization conditions correspond to a higher temperature of 55 ^{O}C, *e.g.*, 40 % formamide, with 5X or 6X SCC. High stringency hybridization conditions correspond to the highest temperature of 65 ^{O}C, *e.g.*, 50 % formamide, 5X or 6X SCC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the

nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m (melting temperature) for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA.

Expression of GnTIII and other Heterologous Proteins in Plant Host Systems

In one embodiment, the nucleic acid encoding the mammalian GnTIII or other heterologous proteins, such as a heterologous glycoprotein or mammalian glycosyltransferase may be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation, as well as selectable markers. These include but are not limited to a promoter region, a signal sequence, 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and transcription and translation termination sequences. Methods for obtaining such vectors are known in the art (see WO 01/29242 for review).

Promoter sequences suitable for expression in plants are described in the art, *e.g.*, WO 91/198696. These include non-constitutive promoters or constitutive promoters, such as, the nopaline synthetase and octopine synthetase promoters, cauliflower mosaic virus (CaMV) 19S and 35S promoters and the figwort mosaic virus (FMV) 35 promoter (U.S. Pat. No. 6,051,753). Promoters used may also be tissue specific promoters targeted for example to the endosperm, aleurone layer, embryo, pericarp, stem, leaves, kernels, tubers, roots, etc.

A signal sequence allows processing and translocation of a protein where appropriate. The signal can be derived from plants or could be non-plant signal sequences. The signal peptides direct the nascent polypeptide to the endoplasmic reticulum, where the polypeptide subsequently undergoes post-translational modification. Signal peptides can routinely be identified by those of skill in the art. They typically have a tripartitite structure, with postively charged amino acids at the N-terminal end, followed by a hydrophobic region and then the cleavage site within a region of reduced hydrophobicity.

The transcription termination is routinely at the opposite end from the transcription initiation regulatory region. It may be associated with the transcriptional initiation region or from a different gene and may be selected to enhance expression. An example is the NOS terminator from Agrobacterium Ti plasmid and the rice α-amylase terminator. Polyadenylation tails may also be added. Examples include but are not limited to Agrobacterium octopine synthetase signal, (Gielen, *et al.*, *EMBO J.* 3:835-846, 1984) or nopaline synthase of the same species (Depicker, *et al.*, *Mol. Appl. Genet.* 1:561-573 , 1982).

Enhancers may be included to increase and/or maximize transcription of the heterologous protein. These include, but are not limited to peptide export signal sequence, codon usage, introns, polyadenylation, and transcription termination sites (see, WO 01 /29242).

Markers include herbicide tolerance and prokaryote selectable markers. Such markers include resistance toward antibiotics such as ampicillin, tetracycline, kanamycin, and spectinomycin. Specific examples include but are not limited to streptomycin phosphotransferase (*spt*) gene coding for streptomycin resistance, neomycin phosphotransferase (*nptII*) gene encoding kanamycin or geneticin resistance, hygromycin phosphotransferase (*hpt*) gene encoding resistance to hygromycin.

The vectors constructed may be introduced into the plant host system using procedures known in the art (reviewed in WO 01/29242 and WO 01/31045). The vectors may be modified to intermediate plant transformation plasmids that contain a region of homology to an *Agrobacterium tumefaciens* vector, a T-DNA border region from *A. tumefaciens*. Alternatively, the vectors used in the methods of the present invention may be *Agrobacterium* vectors. Methods for introducing the vectors include but are not limited to microinjection, velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface and electroporation. The vector may be introduced into a plant cell, tissue or organ. In a specific embodiment, once the presence of a heterologous gene is ascertained, a plant may be regenerated using procedures known in the art.

Uses of Mammalian GnTIII

The expression of mammalian GnTIII leads to bisected N-glycans on glycoproteins. Bisected N-glycans are important for biological activity of some mammalian glycoproteins. In particular, bisected monoclonal antibodies have enhanced ADCC (antibody-dependent cellular cytotoxicity). Introduction of bisected structures leads to new or optimized functionalities and increased bioavailability of glycoprotein, *e.g.*, increasing the antennary type increases half-life because of reduced clearance by the kidney. Accordingly, the invention is directed to a plant host system comprising said heterologous glycoprotein having bisecting oligosaccharides, particularly bisecting GlcNAc residues and methods for producing said glycoprotein.

Furthermore, expression of GnTIII in plants leads to drastic increase of terminal GlcNAc's compared to wildtype plants (plant N-glycans contain far less GlcNAc residues compared to mammalian N-glycans). More GlcNAc residues on N-glycans of plant glycoproteins or recombinant glycoprotein produced in plants resembles mammalian N-glycans of glycoproteins. The introduction of bisected GlcNAc in plant N-glycans (and in plant-produced recombinant glycoproteins such as Mabs) due to GnTIII expression in plants seems to prevent the N-glycan from degradation by β-N-acetylhexosaminidases. More GlcNAc residues means more acceptor substrate for β(1,4)-galactosyltransferase (GalT) adding terminal galactose. Co-expression of GnTIII and a functional protein such as a transporter or a (mammalian) enzyme or functional fragment thereof providing N-glycan biosynthesis, such as a galactosyltransferase, such as GAIT, or crossing GnTIII plants with GalT plants and vice versa, leads to increased galactosylation of glycoproteins s produced in these plants. Accordingly, the invention is directed to a plant host system comprising said mammalian GnTIII and said functional protein; the plant host system may further comprise a heterologous glycoprotein with increased galactosylation relative to a heterologous glycoprotein produced in a

plant host system not comprising said mammalian GnTIII and said functional protein, methods for providing said plant host systems and methods for producing said glycoprotein.

Generating stably transformed plants which produce tailored glycoproteins with commercial interest can be established by inoculating plant cells or tissues with *Agrobacterium* strains containing a vector which comprises both nucleotide sequences encoding GnTIII, optionally N-glycosylation modifying enzymes and nucleotide sequences encoding commercially interesting heterologous glycoproteins or by the procedures described above such as electroporation, microinjection, velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface and electroporation. Alternatively, stably transformed plants which produce tailored glycoproteins with commercial interest can be generated by simultaneous inoculation (cotransformation) of two or more Agrobacterium strains each carrying a vector comprising either nucleotide sequences encoding GNTIII, optionally, other N-glycosylation modifying enzymes or nucleotide sequences encoding glycoproteins of commercial interest or direct cotransformation of plant cells or tissues with said vectors. Alternatively, stably transformed plants which produce tailored glycoproteins with commercial interest can be generated by (multiple) crossing(s) of plants with modified N-glycosylation with plants which express nucleotide sequences encoding proteins of commercial interest. In all of these procedures, the vector may also comprise a nucleotide sequence which confers resistance against a selection agent.

In order to obtain satisfactory expression of the proteins involved in N-glycosylation, GnTIII and of the glycoproteins or polypeptides of commercial interest, the nucleotide sequences may be adapted to the specific transcription and translation machinery of the host plant as known to people skilled in the art. For example, silent mutations in the coding regions may be introduced to improve codon usage and specific promoters may be used to drive expression of the said genes in the relevant plant tissues. Promoters which are developmentally regulated or which can be induced at will, may be used to ensure expression at the appropriate time, for example, only after plant tissues have been harvested from the field and brought into controlled conditions. In all these cases, choice of expression cassettes of the glycosylation modifying proteins and of the glycoproteins of commercial interest should be such that they express in the same cells to allow desired post translational modifications to the said glycoprotein.

As described above, in a specific embodiment, a plant that can be used in the method of the present invention is a tobacco plant, or at least a plant related to the genus *Nicotiana,* however, use for the invention of other relatively easy transformable plants, such as *Arabidopsis thaliana* or *Zea mays,* or plants related thereto, is also particularly provided. For the production of recombinant glycoproteins, the use of duckweed offers specific advantages. The plants are in general small and reproduce asexually through vegetative budding. Nevertheless, most duckweed species have all the tissues and organs of much larger plants including roots, stems, flowers, seeds and fronds. Duckweed can be grown cheaply and very fast as a free floating plant on the surface of simple liquid solutions from which they can easily be harvested. They can also be grown on nutrient-rich waste water, producing valuable products while simultaneously cleaning wastewater for reuse. Particularly

relevant for pharmaceutical applications, duckweed can be grown indoors under contained and controlled conditions. Stably transformed Duckweed can for example be regenerated from tissues or cells after (co)-inoculating with Agrobacterium strains containing each a (binary) vector which comprises one or more nucleotide sequences of interest encoding N-glycosylation modifying enzymes and/or genes encoding commercially interesting heterologous glycoproteins. The duckweed plant may, for example, comprise the genus *Spirodella*, genus *Wolffia*, genus *Wolffiella*, or the genus *Lemna*, *Lemna minor*, *Lemna miniscula* and *Lemna gibba*. Also mosses such as *Physcomitrella patens* offer advantages in that it can be grown cheaply under contained conditions. In addition the haploid genome of *Physcomitrella patens* is relatively easy to manipulate.

Expression in tomato fruits also offers specific advantages. Tomatoes can be easily grown in greenhouses under contained and controlled conditions and tomato fruit biomass can be harvested continuously throughout the year in enormous quantities. The watery fraction containing the glycoproteins of interest can be readily separated from the rest of the tomato fruit which allows easier purification of the glycoprotein. Expression in storage organs of other crops including but not limited to the kernels of corn, the tubers of potato and the seeds of rape seed or sunflower are also attractive alternatives which provide huge biomass in organs for which harvesting and processing technology is in place. Expression in nectar offers specific advantages with respect to purity and homogeneity of the glycoprotein secreted in the nectar.

Alternatively, a plant comprising a heterologous glycoprotein is crossed with a plant according to the invention comprising GnTIII and optionally at least one functional mammalian protein, *e.g.*, a transporter or an enzyme providing N-glycan biosynthesis that is normally not present in plants, harvesting progeny from said crossing and selecting a desired progeny plant expressing said heterologous glycoprotein and expressing GnTIII and optionally a functional (mammalian) enzyme involved in mammalian-like N-glycan biosynthesis that is normally not present in plants. This process is known as crosspollination. In a preferred embodiment, the invention provides a method according to the invention further comprising selecting a desired progeny plant expressing said recombinant protein comprising bisecting oligosaccharide, particularly galactose residues and/or increased galactosylation. Now that such a plant is provided, the invention also provides use of a transgenic plant to produce a desired glycoprotein or functional fragment thereof, in particular wherein said glycoprotein or functional fragment thereof comprises bisecting oligosaccharide and/or increased galactosylation.

The invention additionally provides a method for obtaining a desired glycoprotein or functional fragment thereof comprising cultivating a plant according to the invention until said plant has reached a harvestable stage, for example when sufficient biomass has grown to allow profitable harvesting, followed by harvesting said plant with established techniques known in the art and fractionating said plant with established techniques known in the art to obtain fractionated plant material and at least partly isolating said glycoprotein from said fractionated plant material. The presence of desired proteins may be screened using methods known in the art, preferably using screening assays where the biologically active site is detected in such a way as to produce a

detectable signal. This signal may be produced directly or indirectly. Examples of such assays include ELISA or a radioimmunoassay.

The introduction of bisected GlcNAc residues due to expression of GnTIII can also be used for the prevention of removal (degradation) of saccharides from N-glycan by "blocking" activity glycosidases, *e.g.*, β-N-acetylhexosaminidases and preventing the addition of other saccharides (driven by "other" subsequent glycosyltransferase genes) to N-linked glycan, *e.g.*, fucosylation, xylosylation. By controlling localization (*e.g.*, by providing other subcellular targetting signals) and/or controlling expression levels (*e.g.*, varying levels in independent transgenic plants or using different promoter) glycoform composition could be modulated. Hence introduction of bisecting GlcNAc residues in glycoproteins in plants including recombinant glycoproteins, inhibits incorporation α-1,3-fucose by blocking activity α 1,3fucosyltransferase, α-1,4-fucose by blocking α-1,4-fucosyltransferase, β-1,2-xylose by blocking β-1,2-xylosyltransferase, β-1,3-galactose by blocking β-1,3-galactosyltransferase and removal/degradation of saccharides added to the N-glycan especially terminal GlcNAc residues by blocking activity of β-N-acetylhexosaminidases and terminal β-1,4-galactose (added by expression of β-1,4-galactosyltransferase as provided by patent application WO 01/31045) by blocking β-1,4galactosidase. Thus in this way, controlled expression of GnTIII and controlled introduction of bisecting GlcNAc residues can be used to steer glycoform composition and/or limit glycoform heterogeneity.

Modified GnTIII andGnTIII Hybrid proteins

The invention is further directed to an isolated hybrid protein comprising a catalytic portion of mammalian GnTIII and a transmembrane portion of a protein, said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell. The invention is also directed to a modified mammalian GnTIII, wherein the transmembrane domain is removed but comprising a retention signal such as KDEL for retention of said GnTIII in the ER.

A nucleic acid sequence encoding a hybrid enzyme comprising a transmembrane portion of a first enzyme and a catalytic portion of a second enzyme may be obtained as follows. The sequence encoding the transmembrane portion is removed from the second enzyme, leaving a nucleic acid sequence comprising a nucleic acid sequence encoding the C-terminal portion of the second enzyme, which encompasses the catalytic site. The sequence encoding the transmembrane portion of the first enzyme is isolated or obtained via PCR and ligated to the sequence encoding a sequence comprising the C-terminal portion of the second enzyme.

A nucleic acid sequence encoding a protein, particularly enzymes such as galactosyltransferases, mannosidases and N-acetylglucosamine transferases that are retained in the ER may be obtained by removing the sequence encoding the transmembrane fragment and substituting it for a methionine (initiation of translation) codon and by inserting between the last codon and the stop codon of galactosyltransferase the nucleic acid sequence encoding an ER retention signal such as the sequence encoding KDEL (amino acid residue sequence: lysine-aspartic acid-glutamic acid-leucine) (Rothman, 1987).

Besides controlling expression, relocalization of GnTIII activity may also be controled by making a fusion of the gene sequence coding for the enzymatic part of GnTIII with a transmembrane domain of other glycosyltransferases or enzymes/proteins residing in the endoplasmic reticulum (ER) or Golgi apparatus membrane, or by adding so-called retention signal such as but not limited to KDEL for retention in the ER. Such relocalization modulates the addition of specific saccharides to the N-linked glycan of glycoproteins including recombinant glycoprotein and the prevention of removal of these.

The exchange of transmembrane domain of GnTIII with that of, for example, GnTI (TmGnTI), mannosidase II (TmManII) xylosyltransferase (TmXyl) or α-1,3 fucosyltransferase (TmFuc) but not limited to these, enables earlier expression of GnTIII and introduction of bisecting GlcNAc at positions 20 to 22 in Figure 2. This prevents the action of subsequent glycosyltransferases such as xylosyltransferase and fucosyltransferase to act on the substrate leading to glycoforms lacking Xyl and Fuc. Importantly, the additional of terminal galactose by the action of β-1,4)-galactosyltransferase (GalT) is not inhibited by the bisecting GlcNAc- Co-expression of GalT (Bakker, *et al.*, "Galactose-extended glycans of antibodies produced by transgenic plants" *Proc. Nat. Acad. Sci. USA* 98:2899-2904, 2001) results in structures similar as indicated to the right of the arrows annotated with 20, 21 and 22 in Figure 2. Although devoid of immunogenic xylose and fucose residues, these structures have only one arm processed to complex type glycans. To allow conversion of also the other arm, in addition to relocating GnTIII, also Mannosidase II (ManII) and GnTII are relocated in the Golgi to act earlier in the glycan processing sequence. This can be established in several ways. For example, by exchanging their respective transmembrane domains by that of GnTI (TmGnTI), which results in relocation to position indicated 5 in Figure 2. Alternatively, both MarnII and GnTII can be relocalised to the ER by removing the transmembrane Golgi targeting domain and supplying the remaining enzyme fragments with a C-terminal ER retention signal (*e.g.*, the amino acid residues KDEL). A plant expressing GalT (Bakker, *et al.*, "Galactose-extended glycans of antibodies produced by transgenic plants" *Proc. Nat. Acad. Sci. USA* 98:2899-2904, 2001) as well as the relocated versions of GnTIII (*e.g.*, TmXyl-GnTIII), ManII (*e.g.*, TmGnTI-ManII) and GnTII (*e.g.*, TmGnTI-GnTII) can than be crossed with plants expressing the recombinant glycoprotein of interest (Figure 3) or can be retransformed with the gene encoding the glycoprotein of interest such as the genes encoding an antibody. This allows the production of recombinant glycoproteins having bisected glycans with terminal galactose residues which are devoid of xylose and fucose. Transformation procedures and crossing (co-pollination) procedures are described above.

In another embodiment, GnTIII with transmembrane domain of Mannosidase II(TmManII-GnTIII) or xylosyltransferase (Tmxyl-GnTIII) combined with TmXyl-GalT, TmGnTI-GnTII, TmGnTI-ManII. This combination could either be obtained by coexpression or by combining through cross-pollination of the genes involved and leads to glycoproteins including recombinant glycoproteins, lacking xylose and fucose on the core sequence but having bisected GlcNAc residues on the trimannosyl core and terminal galactose.

EXAMPLES

The effect of the introduction of GnTIII in plants on the occurrence of bisected oligosaccharides on the glycans of plant glycoproteins has been evaluated. The human gene for GnTIII has been cloned; and a C-terminal c-myc tag for analysis of expression of the tagged fusion protein has been provided and the whole has been placed under control of plant regulatory elements for introduction in tobacco. It is shown that GnTIII is expressed in plants and that expression results in bisected oligosaccharide structures on endogenous plant glycoproteins. The amount of N-glycans containing at least two G1cNAc residues more than doubled compared to those found in normal tobacco plants. Remarkably, the expression of GnTIII also resulted in a significant reduction of complex type N-glycan degradation products as apparent from matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analyses of the isolated glycans of endogenous plant glycoproteins. These data suggest that expression of GnTIII in tobacco resulting in the introduction of bisected structures on N-glycans protects the glycans from degradation by β-N-acetylhexosaminidases. β-N-acetylhexosaminidases have broad specificity for non-reducing terminal G1cNAc and β-N-acetylglucosamine (GalNAC) cleaving amongst others G1cNAc-β1-2 linkages typically present on the trimannosyl core (Man-α-l-3 and Man-α-l-6).

Example 1

Plasmids and plant transformation. PAC clone RP5-1104E15 GnTIII (SEQ ID NO: 1, Figure 4A) was obtained from Pieter J. de Jong, Children's Hospital Oakland Research Institute (CHORI) and is available on request through Sanger Center being part of clone set HBRC_1.sc. The clone originates from Homo sapiens, male, blood and can be requested through http://www.sanger.ac.uk/Teams/Team63/CloneRequest/ (from Human chromosome 22q12.3-13.1; The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK; www.sanger.ac.uk)

The human gene for GnTIII was cloned from said PAC clone by PCR using AccuTaq LA DNA polymerase (SigmaAldrich) and primers GNT3F (5'atactcgagttaacaatgaagatgagacgct-3'; SEQ ID NO: 3) and GNT3Rmyc (5'-tatggatcctaattcagatcctcttctgagatgag-3'; SEQ ID NO: 4). Oligos were from Eurogentec (Belgium). PCR was performed on a PerkinElmerCetus 480 thermal cycler (ABI/PE) using optimal conditions for the AccuTaq polymerase according to the manufacturer. The resulting fragment was cloned in EcoRV site of pBluescribe SK+ (Stratagene, Inc., La Jolla, CA USA) and sequence verified. Sequencing was performed using fluorescently labelled dideoxynucleotides essentially as described (Sanger, *et al.*, "DNA sequencing with the dideoxy chain-terminating inhibitors" *Proc. Nat. Acad. Sci. USA* 74:5463-5467, 1977) and reaction mixtures were run on an Applied Biosystems 370A or 380 automated DNA sequencer. Data were analysed using different software modules freely available on the web and compared with the DNA sequence of human GnTIII present in the database.

A 1.6 kb HpaI/BamHI fragment containing the GnTIII gene with C-terminal c-myc tag was

subsequently cloned into the Sma/BglII site of pUCAP35S (Van Engelen, *et al.*, "Coordinate expression of antibody subunit genes yields high levels of functional antibodies in roots of transgenic tobacco" *Plant Molecular Biology* 26:1701-1710, 1994) and named pAMV-GnTIII. The cauliflower mosaic virus 35S (CaMV35S) 20 promoter expression cassette with modified GnTIII gene was subsequently cloned as a AscI/PacI fragment in the binary vector pBINPLUS (Van Engelen, *et al.*, "Coordinate expression of antibody subunit genes yields high levels of functional antibodies in roots of transgenic tobacco" *Plant Molecular Biology* 26:1701-1710, 1994) resulting in pBINPLUSGnTIII and introduced in *Agrobacterium tumefaciens* strain Ag10 by electroporation. Transformation *of Nicotiana tabacum* variety Samsun NN was as described before (Horsch, *et al.*, "A simple and general method for transferring genes into plants" *Science* 227:1229-1231, 1985). Sixteen independent transgenic plants were selected and grown to maturity in the greenhouse as described. Leaf material was analysed for expression of GnTIII (SEQ ID NO: 2, Figure 4B) and glycan composition of endogenous cellular glycoproteins.

Example 2

Analysis of expression. Total protein extracts of tobacco leaves were prepared as described before (Bakker, *et al.*, "Galactose-extended glycans of antibodies produced by transgenic plants" *Proc. Nat. Acad. Sci. USA* 98:2899-2904, 2001). The amount of protein present in samples was estimated by the Bradford method (Bradford, M.M., "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding" *Anal Biochem* 72:248-254, 1976) using bovine serum albumin as standard. Fixed amounts *of* protein samples were run on precast 10 or 12 % SDS-PAGE gels (Bio-Rad) under reduced conditions. Rainbow coloured molecular weight protein markers were from Amersham. Western blot analysis was performed essentially as described (Bakker, *et al.*, "Galactose-extended glycans of antibodies produced by transgenic plants" *Proc. Nat. Acad. Sci. USA* 98:2899-2904, 2001). Separated proteins were transferred to nitrocellulose (BA85, Schleicher and Schuell or Trans-Blot Transfer Medium, Bio-Rad) using a Bio-Rad Mini Trans-blot Electrophoretic Transfer Cell in 3[cyclohexylamino]-1-propanesulfonic acid (CAPS) buffer for 60 min. Expression of the GnTIII-c-myc fusion protein was analysed by affinoblotting using a peroxidase labelled c-myc antibody. Introduction of bisecting oligosaccharides in endogenous tobacco glycoproteins was visualized by incubation with biotinylated erythroagglutinating phytohemagglutinin (E-PHA; Vector Laboratories). Detection was performed by enhanced chemiluminescence using Lumi-Light Western Blotting Substrate from Roche (Roche Diagnostics GmbH, Mannheim, Germany) on a Lumi-Imager F 1 apparatus (Boehringer Mannheim GmbH, Mannheim, Germany) using LumiAnalyst software (version 3.0).

Example 3

Matrix-assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry. For the analysis of glycan structure cellular proteins were isolated from tobacco leaves of a selected plant transformed with human GnTIII (GnTIII-17). Protein isolation and N-

glycan preparation were performed as described (Elbers, *et.al.*, 2001). The N-glycans were desalted on a nonporous, graphitized carbon-black column (Carbograph Ultra Clean Tubes, Alltech Associates) before mass spectrometry analysis as described. MALDI-TOF spectra were measured on a Micromass (Manchester, U.K.) Tof spec E MALDI-TOF mass spectrometer. Mass spectra were performed in positive mode by using 2,5-dihydroxybenzoic acid as the matrix essentially as described (Elbers, *et al.*, 2001).

Expression of human GnTIII introduces bisecting N-glycans on endogenous glycoproteins in *N. tabacum*. Human GnTIII was introduced in tobacco plants by *Agrobacterium*-mediated transformation of binary vector pBINPLUSGnTIII containing a cDNA harbouring the complete coding sequence fused to a C-terminal c-myc tag under control of the constitutive CaMV35S promoter. Sixty independent transgenic shoots selected for kanamycin resistance were obtained which were analysed for expression of the GnTIII-c-myc fusion protein using the c-myc antibody. Analysis revealed that all expressed the gene at various levels. Fourteen were selected, rooted and transferred to the greenhouse. One plant (GnTIII-17) selected for high expression of the GnTIII-c-myc fusion protein using the c-myc antibody was analysed for the occurrence of bisected GlcNAc residues on N-glycans of endogenous tobacco glycoproteins using a specific binding assay with the biotinylated lectin E-PHA. SDS-PAGE of protein extracts followed by transfer to nitrocellulose and analysis using the specific binding assay with the biotinylated E-PHA lectin revealed that endogenous tobacco glycoproteins of GnTIII-17 contained bisected oligosaccharides whereas those of control tobacco did not. GnTIII-17 was multiplied in the greenhouse for further detailed analysis of glycan structure by MALDI-TOF.

Example 4

Oligosaccharide distributions and level of bisected complex oligosaccharides in wildtype and selected transgenic GnTIII-17 tobacco plant. Endogenous glycoproteins were isolated from young leaves of control tobacco plant and the selected GnTIII-17 plant to investigate in detail the effect of expression of human GnTIII on the structure of glycans N-linked to glycoproteins. A comparison of the structures of the N-glycans isolated from glycoproteins present in leaves of control wild-type tobacco plants with those from plant GnTIII-17 using MALDI-TOF is represented in Figure 1. MALDI-TOF allows for the detection of different molecular species in the pool of the N-glycans (glycoforms) and shows a mixture of ions that were assigned to (M+Na)+ adducts of high-mannose (Man)- type N-glycans ranging from **d**, Mans to **n**, Man9 and of mature N-glycans from the truncated structure **a**, XM3GN2 to **m**, GN3FXM3GN2 (for structure see Table 1; for a summary of the data see, Table 2). In addition to the N-glycans characterized in the control plants (Figure 1A), the MALDI-TOF MS of the glycan mixture from plant GnTIII-17 (Figure 1B) showed at least two ions assigned to N-linked glycans that result from the action of the human GnTIII enzyme (for a comparison see Table 1 and Table 2). These oligosaccharides, GN3XM3GN2 (**i**) and GN3FXM3GN2 (**k**) representing 8 % and 31 % respectively of the population, contain three GlcNAc residues each linked to one of the three mannoses of the trimannosyl core structure of the

N-linked glycan.

Analysis of glycan structure through MALDI-TOF as performed here cannot distinguish between GlcNAc residues β(1,2)- or β(1,4)-linked to mannose. Hence, it was not clear if or to what extent the structures GN2XM3GN2 and GN2FXM3GN2 have bisecting oligosaccharides. Additional experiments are required to reveal that these structures are a mix of normal and bisected oligosaccharides or a single compound.

In the light of the observed lethality of CHO cell that overexpress GnTIII (Umana, *et al.*, "Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity" *Nature Biotechnology* 17:176-180, 1999), remarkably transgenic plants having significant amounts of bisected glycans look phenotypically normal and are completely fertile (can be cross-pollinated and self-pollinated).

Example 5

Expression of human GnTIII in tobacco seems to protect N-glycans from degradation by D-Nacetylhexosaminidases and more than doubles terminal N-glucosaminylation. MALDI-TOF analysis of extracts clearly showed that at least 40 % of the population of glycoforms now has a bisecting GlcNAc in complex-type N-linked glycans of cellular tobacco proteins through the action of the GnTIII enzyme. Moreover 70 % of the population of complex-type N-linked glycans of endogenous glycoproteins of GnTIII-17 has two or three terminal GlcNAc residues compared to about 30 % for wildtype tobacco (Table 1). The observed *de novo* synthesis of at least 40 % bisected complex-type N-linked glycans upon expression of GnTIII in tobacco (Figure 1B,Table 1 and Table 2) coincides with the disappearance of mainly FXM3GN2 (b, from 30 % to 4 %) and GNFXM3GN2 (f, from 10 to 2%) and to a minor degree GN2FXM3GN2 (j, from 29 % to 19 %). In addition it also coincides with a significant increase in GN2XM3GN2 (h) from 4 % in wildtype tobacco to 14 % in GnTIII-17. Whether the latter GN2XM3GN2 (h) in GnTIII plants has the second GlcNAc linked to the (β-linked mannose of the trimannosyl core of the N-linked glycan and hence is the result of GnTIII activity, or to the second α-linked mannose of the trimannosyl core remains to be investigated (see above).

Saccharides a, b and c accounting for 40 % of the N-linked glycans in wildtype tobacco plants, are degradation products expected to have arisen from mature glycans of endogenous tobacco glycoproteins after GnTI activity since an Arabidopsis thaliana mutant lacking GnTI activity did not contain xylose and fucose residues in the N-glycans of endogenous glycoproteins (Von Schaewen, *et al.*, "Isolation of a mutant Arabidopsis plant that lacks N-Acetylglucosaminyl transferase I and is unable to synthesize golgi-modified complex N-linked glycans" *Plant Physiology* 102:1109-1118, 1993). The 7-fold decrease (40 % > 6 %) in these structures in GnTIII-17 together with the threefold reduction of GNXM3GN2 and XM3GN2 (12 % > 4 %) suggests that the introduction of a bisected GlcNAc protects the mature N-linked glycan from degradation by endogenous glycosidases especially β-Nacetylhexosaminidases that removes terminal GlcNAc. The total amount of N-linked glycans expected to have arisen from degradation of mature, full-length N-linked glycans has hence

decreased fivefold (from 52 % to 10 %).

Example 6

Vector construction and DNA preparation for maize transformation. The human GNTIII gene along with its 3' c-myc immunodetection tag was obtained by PCR from plasmid pAMV-GNTIII by the following method. Primers MS20 and MS19 homologous to the 5' and 3' ends of the hGNTIII gene respectively, were designed and synthesized to add a Pme1 site and a stop codon to the 3' end of the gene.

MS20 (5' *Nco1* site): 5'-CCATGGTGATGAGACGCTAC-3' (SEQ ID NO: 5)
MS19 (adds stop and *Pme1* site 3'): 5'-GTTTAAACCTAGGATCCTAATTCAGATCCTCT-3' (SEQ ID NO: 6)

Following gel electrophoresis to identify the correct sized PCR product, the 1.6 kbp PCR product was recovered from the gel with a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Plasmid 4005 (see, SEQ ID NO: 8) (Figures 5A and 5B), which contains a Zmubi /GUS /per5 cassette (Christensen, *et al.*, *Plant Molec. Biol.* 18:675-689, 1992), was digested with Nco1 and Pme1 to release the GUS gene and the vector fragment was recovered from a gel with a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA).

Following digestion with Nco1 and Pme1, the PCR-derived hGNTIII fragment was ligated to the vector fragment left after digestion of pDAB4005 with Nco1 and Pme1, to create the intermediate plasmid pDAB7119 (see, SEQ ID NO: 9) (Figures 6A and 6B). Intermediate plasmid pDAB7119 was cut with Spe1 and Sph1 to release the hGNTIII plant expression cassette, which was treated with T4 DNA polymerase to create blunt ends.

Plasmid pDAB8504 (SEQ ID NO: 10) (see, Figures 7A and 7B), which contains the RB7 MAR sequences, was digested with Srf1 and blunt ended with T4 DNA polymerase. Following treatment with calf intestinal phosphatase, the treated 8504 fragment and the hGNTIII plant expression cassette were ligated to create plasmid pDAB 7113 (SEQ ID NO: 10) (see, Figures 8A and 8B), which contains RB7 MAR sequences flanking the gene of interest and the selectable marker cassete as follows: RB7 MAR // Zmubi promoter/hGNTIII/per5 3'UTR // Rice actin promoter (D. McElroy, *et al.*, "Isolation of an efficient actin promoter for use in rice transformation" *The Plant Cell* 2:163-171, 1990) /PAT/ Zm lipase 3'UTR // RB7 MAR.

The integrity of the GNTIII sequence was checked by sequencing (Big Dye Terminator Cycle Sequencing Ready Reaction, Applied Biosystems, Foster City, CA) and was confirmed to encode the human GNTIII enzyme. One base change, G384 → 384, was found but this substitution does not affect the encoded amino acid, proline 128.

Plasmid pDAB7113 was grown up in 2 L of medium (LB + amp) and purified with Qiagen plasmid Giga kit to produce 5 milligrams of purified plasmid for plant cell transformation.

Example 7

Transformation of maize cells. Plasmid pDAB7113 was introduced into maize cells with WHISKERS-mediated DNA transfer essentially as described in these citations, and as follows (Frame, B., *et al.*, "Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation" *Plant J.* 6:941-948, 1994; Thompson, J., *et al.*, "Maize transformation utilizing silicon carbide whiskers: a review" *Euphytica* 85:75-80, 1995; P. Song, C. Q. Cai, M. Skokut, B. Kosegi, and J. Petolino, "Quantitative real-time PCR as a screening tool for estimating transgene copy number in Whiskers-derived transgenic maize" *Plant Cell Rep.* 20:948-954, 2002; both of which are incorporated herein by reference).

Embryogenic maize suspension cell cultures were subcultured on medium G-N6 (N6 medium containing 30 gm/L sucrose, 100 mg/L inositol, and 2 mg/L 2,4-D) the day before whisker mediated transformation. On the day of the experiment, cells were pretreated with osmoticum by shaking with medium G-N6 containing 0.2 Molar each mannitol and sorbitol for 30 minutes. Thirty six mls of cells were transferred to a 250 ml centrifuge bottle in 50 ml of medium G-N6, to which was added 8.1 ml of a 5 % (w/v) silicon carbide whiskers suspension (Silar SC-9, Advanced Composite Materials, Greer, S.C.) in medium, plus 170 ul of 1 mg/ml plasmid solution (in TE buffer). The centrifuge bottle containing cells, whiskers and DNA was agitated vigorously on a modified Red Devil brand paint mixer for 10 seconds. Whiskered cells were then shaken for two hours in medium with half the level of added osmoticum. Whiskered cells were recovered by filtration on a sterile Buchner funnel and the filter papers were placed on semisolid G-N6 medium for 1 week. After 1 week the filters were moved to semisolid G-N6 medium containing 1 mg/L Herbiace (a commercial formulation of 20% bialaphos, Meiji Seika, Tokyo, Japan). Two weeks later, the cells were removed from the filter paper, mixed with melted G-N6 + 1 mg/L Herbiace (G-N6 +1H) medium also containing 7 gm/L Seaplaque agarose (BioWhittaker, Rockland, Maine), and spread on top of G-N6 + 1H solid medium. Plates were cultured in the dark at 30 ^{O}C. Colonies resistant to the selective agent were recovered 5-7 weeks post embedding, and individually moved to fresh G-N6+1H medium for further increase of tissue mass.

Example 8

Molecular analysis for copy number of inserted DNA. Tissue from each transgenic isolate was individually freeze-dried in a lyophilizer and DNA was extracted by a standard method (DNAeasy 96 Plant Kit, Qiagen). The copy number of inserted transgenic DNA was estimated by the Invader Operating System, available from Third Wave Technologies (Third Wave Technologies, Madison, Wisconsin, twt.com). Primers were designed by the Third Wave Technologies company specifically for the PAT selectable marker and its copy number was estimated relative to genomic DNA copy number for the endogenous maize alpha-tubulin gene.

Example 9

Test transgenic maize callus for altered lectin binding due to expression of the GntIII

gene. Callus samples from 100 individually isolated unique transgenic events were extracted as follows. Samples from each event were fresh frozen in 96-well cluster tube boxes (Costar 1.2 ml polypropylene, with lid) along with a steel and a tungsten bead in each well. 450 ul of extraction buffer (25 mM sodium phosphate pH6.6, 100 mM NaCl, 30 mM sodium bisulfate, 1 % v/v Triton X-100) was added per well and the box of samples was pulverized for 3 minutes full speed on a Kleco Bead Mill. The plate was centrifuged (4 ^{O}C) at 2500 rpm for 10 minutes. Extracts were removed to a 96-well deep well plate and frozen for storage. All screening assays were performed on these extracts of individual events.

Protein analyses (microtiter plate protocol, BioRad 500-0006) were made to determine the total protein for each extract. 25 ug protein per sample were loaded in 20 ul loading buffer (Laemmli, U.K. Nature 277:680 (1970)). Gels (4-20 % Criterion PAGE gels, 12+2 wells, BioRad 345-0032) were electrophoresed at 65 mA in Tris/glycine /SDS running buffer (BioRad 161-077). After soaking in transfer buffer (running buffer plus 20 % v/v methanol) for 10 minutes, the gels were transferred to nitrocellulose membranes using a semi-dry blotter (150 mA/1.5 hrs). The membranes were incubated for 30 minutes in blocking buffer (20 mM Tris, 144 mM NaCl, 0.5 % v/v Tween 20, 10 % w/v nonfat powdered milk) at room temperature, then the blocking buffer was removed and replaced with the primary detection lectin (Phaseolus hemagglutinin E, biotinylated, Vector Laboratories B-1125) 2.5 ug/ml in blocking buffer. The primary detection lectin was incubated on the membrane for 1 hour at room temperature. The primary detection solution was removed, the membrane was rinsed once with blocking buffer and the secondary detection solution was added (avidin-HRP, BioRad 170-6528, at 1:5000, plus molecular weight marker detection agent, StrepTactin-HRP, BioRad 161-0380 at 1:10,000 in blocking buffer. The secondary detection reagent was incubated on the membrane for 1 hour at room temperature. During the blocking, primary, and secondary reagent steps the solutions were mixed on the blots. The secondary detection reagent was then removed and the membrane was rinsed with Tris buffered saline (20 mM Tris, 144 mM NaCl) containing 0.5 % Tween 20 three times at 10 minutes each and once more for 5 minutes. After dripping off the excess rinse solution, the blot was soaked in substrate ECL (Pierce 34080) for 1 minute, excess ECL solution was drained off, and the membrane was exposed to film. Negative controls were included in each gel to discriminate new glycoprotein bands now visible with this bisecting glycan -detecting lectin reagent on the transgenic callus extracts.

Positive test results (Table 5) for the E-PHA binding were rated as 0 (negative), 1 (one plus, weak) 2 (two pluses, moderately strong) or 3 (three pluses, strongest rating). Callus of events rated 2 or 3 were selected to produce sample for mass analysis. Samples 25, 26, 33, 48, 55, 56 and 59 were pooled to produce the protein extract for MALDI-TOF analysis of glycan substructures. A gel blot example (Figure 12) shows samples 19 through 27.

Example 10

Test transgenic maize callus for c-myc epitope expression. Callus samples from 100 individually isolated unique transgenic events were extracted as follows. Samples from each event

were fresh frozen in 96-well cluster tube boxes (Costar 1.2 ml polypropylene, with lid) along with a steel and a tungsten bead in each well. 450 ul of extraction buffer (25 mM sodium phosphate pH6.6, 100 mM NaCl, 30 mM sodium bisulfate, 1 % v/v Triton X-100) was added per well and the box of samples was pulverized for 3 minutes full speed on a Kleco Bead Mill. The plate was centrifuged (4 ^{O}C) at 2500 rpm for 10 minutes. Extracts were removed to a 96-well deep well plate and frozen for storage. All screening assays were performed on these extracts of individual events.

Protein analyses (microtiter plate protocol, BioRad 500-0006) were made to determine the total protein for each extract. 25 ug protein per sample were loaded in 20 ul loading buffer (Laemmli, U.K. Nature 277:680, 1970)). Gels (4-20 % Criterion PAGE gels, 12+2 wells per gel, BioRad 345-0032) were electrophoresed at 65 mA in Tris_/glycine /SDS running buffer (BioRad 161-0772). After soaking in transfer buffer (running buffer plus 20 % methanol) for 10 minutes, the gels were transferred to nitrocellulose membranes using a semi-dry blotter (150 mA/1.5 hrs). The membranes were incubated for 30 minutes in blocking buffer (20 mM Tris, 144 mM NaCl 0.5 % v/v Tween 20, 10 % w/v dry milk) at room temperature, then the blocking buffer was removed and replaced with the primary detection reagent, Mouse anti-c-myc clone 9E10 (sigma M5546) at 1 ug/ml in blocking buffer. After 1 hour of incubation at room temperature, the primary detection reagent was removed and the membrane was rinsed with blocking buffer. The sécondary detection reagent, anti-mouse - HRP (BioRad 170-6516) at 1:10,000 plus a molecular weight marker detection reagent (StrepTactin - HRP, BioRad 161-0380) at 1:10,000 in blcking buffer, was then added and incubated on the membrane for 1 hour at room temperature. During the blocking, primary, and secondary reagent steps the solutions were mixed on the blots. The secondary detection agent was removed, and the membrane was rinsed three times with Tris buffered saline (20 mM Tris, 144 mM NaCl) containing 0.5 % Tween 20 for 10 minutes each, plus another 5 minute rinse. After draining off the excess rinse solution the membrane was soaked in ECL reagent (Pierce 34080) for 1 minute, drained, and then exposed to film.

As detailed above, callus samples from independent events 1-100 were screened for expression of the c-myc epitope. Then, samples 3, 11, 12, 26, 31, 55 and 64 were analysed and showed the presence of a band in the predicted molecular weight range of 50-55 kilodaltons. These callus samples were pooled to produce a protein sample for glycan analysis by MALDI-TOF. A representative blot is shown in Figure 13.

Example 11

Preparation of extract for mass spec analysis of glycans. The samples were prepared from combined calluses of several maize callus events which tested positive for GnTIII transgene expression based on lectin blotting using E-PHA. Callus tissue was collected fresh and stored frozen at –80 ^{O}C, then ground to a fine powder in liquid nitrogen. Weighed sample was added to extraction buffer (5 mM EDTA, 0.5 mM PMSF, 20 mM sodium bisulfite, 150 mM sodium phosphate buffer pH 7.4, and 0.4 mM PVPP soluble MW 40,000) and stirred for 30 minutes at 4 ^{O}C. After centrifugation at 5000 x G at 4 ^{O}C, the supernatant was collected. Ammonium sulfate and wash buffer (5 mM

EDTA, 150 mM sodium phosphate buffer, pH 7.4) were added to the supernatant to achieve a final concentration of 20 % (w/v) ammonium sulfate. After centrifugation 5 minutes at 5000 x G at 4 ^{O}C, the supernatant was transferred to a fresh tube and additional ammonium sulfate plus wash buffer were added to achieve 60 % (w/v) ammonium sulfate. This preparation was stirred overnight at 4 ^{O}C, then centrifuged 20 minutes at 10,000 x G . The pellet was recovered in 5 ml of wash buffer and frozen at –80 ^{O}C, then lyophilized at 4 ^{O}C until dry. Samples were sent to the lab for glycan analysis.

Example 12

Maize plant regeneration from transgenic callus tissue. For plant regeneration from transformed callus, tissue was placed onto regeneration media containing MS basal salts and vitamins (Murashige T. and F. Skoog , *Physiol Plant* 15:473-497, 1962), 30 g/l sucrose, 5 mg/l 6-benzylaminopurine (BA), 0.025 mg/l 2, 4-dichlorophenoxyacetic acid (2,4-D), 1 mg/l Herbiace (a commercial formulation of 20 % bialaphos, Meiji Seika, Tokyo, Japan), and 2.5 g/l Gelrite, pH 5.7. Cultures were grown in the light. When shoots reached 1-3 cm in length, they were transferred into vessels containing SH basal salts and vitamins (Schenk R. and A.C. Hildebrandt, *Can J Bot* 50:199-204, 1972), 10 g/l sucrose, 1 g/l myo-inositol, and 2.5 g/l Gelrite, pH 5.8.

Plants were screened for expression of GNTIII by altered binding of the lectin E-PHA to endogenous proteins. Samples were then screened for E-PHA binding as described in Example 9, *supra*. The protein extract and 20 % / 60 % ammonium sulfate precipitate was prepared exactly as for the callus samples as described in Example 13, *infra*. One plant each from plants regenerated from 23 independent events were screened by lectin blotting for the results of expression of the GNTIII gene. Four of these events gave positive signals for E-PHA binding. These four events had also tested positive at the callus stage. Plants regenerated from those four events were pooled to produce a protein extract for glycan analysis by MALDI-TOF.

Example 13

Oligosaccharide distributions and level of bisected complex oligosaccharides in wildtype and selected transgenic corn calli. Endogenous glycoproteins were isolated from control corn calli and selected corn calli expressing GnTIII based on lectin blotting using E-PHA. In addition, the present invention also contemplates the extraction of c-myc tagged samples. E-PHA and c-myc tagged samples may be callus, plant cells, plant tissues or entire plants as defined in the definitions section *supra*. A comparison of the structures of the N-glycans isolated from glycoproteins present in calli is presented in Figures 9A and 9B. MALDI-TOF allowed for the detection of different molecular species in the pool of the N-glycans (glycoforms) and showed a mixture of ions that were assigned to (M+Na)+ adducts of high-mannose (Man)- type N-glycans ranging from **d**, Man5 to **l**, Man8 and of mature N-glycans from the truncated structure **a**, XM3GN2 to **m**, bGN3FXM3GN2 (see, Table 3). In addition to the N-glycans characterized in the control callus (Figure 9A), the MALDI-TOF MS of the glycan mixture from selected corn calli expressing GnTIII (Figure 9B) showed at least one ion assigned to N-linked glycans that result from the action of the human GnTIII

enzyme (for a comparison see, Table 3). This oligosaccharide, GN3XM3GN2 (**m**) represents 20 % of the population and contains three GlcNAc residues each linked to one of the three mannoses of the trimannosyl core structure of the N-linked glycan. Analysis of glycan structure through MALDI-TOF as performed here cannot distinguish between GlcNAc residues β(1,2)- or β(1,4)-linked to mannose. Hence it is not clear if or to what extent the structures GN2XM3GN2 (**h**) and GN2FXM3GN2 (**k**) have bisecting oligosaccharides. Both had increased numbers in GnTIII corn cells compared to untransformed control corn cells. Additional experiments are required to reveal that these structures are a mix of normal and bisected oligosaccharides or a single compound.

Besides the new appearance of saccharide structure **m** (bGN3FXM3GN2) in GnTIII corn, it is apparent from the comparison of the glycoforms of control and GnTIII corn, as shown in Table 3, that the amount of structures harbouring high-mannose type N-glycans (M4 and higher) is reduced more than twofold (from 19 % to 7 %) which can be attibuted mostly to the reduction of M4-containing N-glycans (from 10 % to 1 % of total) in GnTIII corn versus control corn. In addition the amount of glycoforms having two or more GlcNAc residues has increased from 16 % to 42 % (control versus GnTIII).

In a follow-up experiment, endogenous glycoproteins were isolated from control corn calli and selected corn calli expressing GnTIII based on analysis for the presence of c-myc tag sequence by Western blotting. A comparison of the structures of the N-glycans isolated from glycoproteins present in calli is presented in Table 4. MALDI-TOF allows for the detection of different molecular species in the pool of the N-glycans (glycoforms) and shows a mixture of ions that were assigned to (M+Na)+ adducts of high-mannose (Man)- type N-glycans ranging from **d**, Man5 to **l**, Man8 and of mature N-glycans from the truncated structure **a**, XM3GN2 to **k**, GN2FXM3GN2 in control corn.

Remarkably, in transgenic corn expressing GnTIII (Table 4, GnTIII-2), only three isoforms could be detected: FXM3GN2 (**b**; accounting for 9 % of total), GN2FXM3GN2/bGN2FXM3GN2 (**k**; 38 %) and bGN3FXM3GN2 (**m**; 54 %). It is not clear if or to what extent the structure depicted as **k** (GN2FXM3GN2/bGN2FXM3GN2) has bisecting oligosaccharides. Its presence is significantly increased in GnTIII corn compared to control corn. Additional experiments are required to reveal that these structures are a mix of normal and bisected oligosaccharides or a single compound.

Besides the new appearance of saccharide structure m (bGN3FXM3GN2) in GnTIII corn (54 %), it is apparent from the comparison of the glycoforms of control and GnTIII corn, as summarized in Table 4, that the amount of structures harbouring high-mannose type N-glycans (M4 and higher) is reduced to nil in GnTIII corn versus control corn. Furthermore, tha total amount of N-glycans bearing 2 or more (3) GlcNAc residues has increased from 16 to 92% (control versus GnTIII) suggesting that the introduction of bisected GlcNAc residue protects the glycan from degradation by endogenous hexosaminidases as observed before for transgenic GnTIII tobacco.

Additioanlly, MALDI-TOF mass spcetroscopy data (Figure 11) demonstrate the bisected GlcNAc structure.

Example 14

Oligosaccharide distributions and level of bisected complex oligosaccharides in wildtype and selected transgenic corn plants. Endogenous glycoproteins were isolated from control corn plant leaves and selected corn plant leaves expressing GnTIII based on analysis for the presence of c-myc tag sequence by Western blotting or lectin blotting using E-PHA. A comparison of the structures of the N-glycans isolated from glycoproteins present in leaves is presented in Table 6. MALDI-TOF allows for the detection of different molecular species in the pool of the N-glycans (glycoforms) and shows a mixture of ions that were assigned to (M+Na)+ or (M+K)+ adducts of high-mannose (Man)-type N-glycans ranging from **f**, Man6 to **h**, Man8 and of mature N-glycans from the truncated structure **a**, XM3GN2 to **g**, GN2FXM3GN2 in control corn plant and to **i**, bGN3FXM3GN2 in transgenic GnTIII corn plant.

In addition to the N-glycans characterized in the control plants (Figure A), the MALDI-TOF MS of the glycan mixture from selected corn plant expressing GnTIII (Figure B) showed at least one ion assigned to N-linked glycans that result from the action of the human GnTIII enzyme (for a comparison see Table 6). This oligosaccharide, GN3XM3GN2 (i) represents 15% of the population and contains three GlcNAc residues each linked to one of the three mannoses of the trimannosyl core structure of the N-linked glycan. Analysis of glycan structure through MALDI-TOF as performed here cannot distinguish between GlcNAc residues β(1,2)- or β(1,4)-linked to mannose. Hence it is not clear if or to what extent the structure GN2FXM3GN2 (g) has bisecting oligosaccharides. It has increased in GnTIII corn compared to control corn plant (23 % versus 5 % in control). Additional experiments are required to reveal that these structures are a mix of normal and bisected oligosaccharides or a single compound. Besides this it is apparent from the comparison of the glycoforms of control and GnTIII corn plants, as depicted in Table 6, that the amount of structures harbouring FXM3GN2 is reduced twofold (from 59 to 30) and the amount of glycoforms having two or more GlcNAc residues has increased from 5 to 38 % (control versus GnTIII).

Additionally, Figure 14 shows a comparison of MALDI-TOF mass spectra of N-glycans of glycoproteins isolated from leaves of control corn (A) and of selected GnTIII-corn plants. GnTIII corn plant was obtained through transformation with human GnTIII gene sequence and selection was performed by Western blotting using either c-myc tag or E-PHA lectin. See Table 6 for structures and abbreviations.

It is understood that the present invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagent, etc., described herein, as these may vary. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only, and is not intend to limit the scope of the present invention. It must be noted that as used herein and in the appended claims, the singular forms "a ," "an" and "the" include plural reference unless the context clearly dictates otherwise.

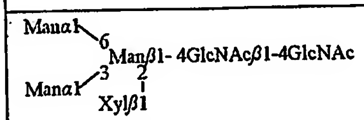
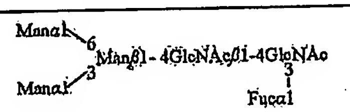
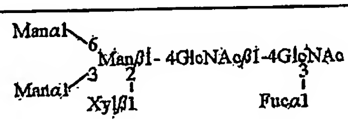
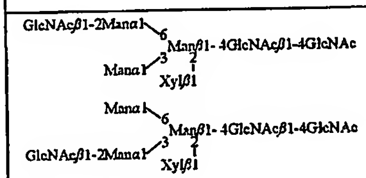
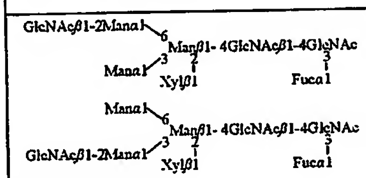
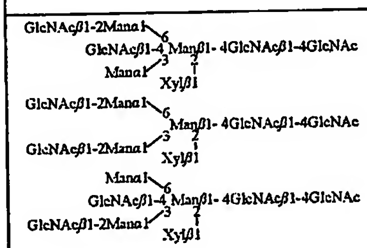
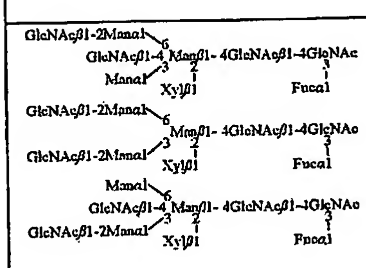
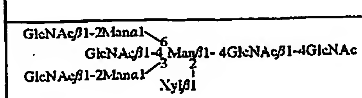
Unless defined otherwise, all technical and scientific terms used herein have the same

meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

Table 1. Structure, molecular weight and percentage of total pool of N-glycans isolated from control and selected GnTIII-17 plants.

Structure	Abbreviation	Name	Mol. Wt.	% Wildtype	% GnTIII-17
	XM3GN2	A	1065	8	4
	FM3GN2	B	1079	3	0
	FXM3GN2	C	1211	30	4
	Man5	D	1257	2	3
	GNXM3GN2	E	1268	4	0
	GNFXM3GN2	F	1414	10	2
	Man6	G	1419	3	5
	GN2XM3GN2	H	1471	- 4 -	14
	Man7	I	1581	3	4
	GN2FXM3GN2	J	1617	- 29 -	19
	GN3XM3GN2	K	1674	-	8

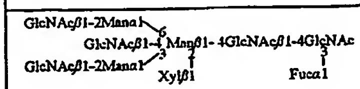
	Man8	L	1743	2	4
	GN3FXM3GN2	M	1820	-	31
	Man9	N	1905	1	2

Table 2. Comparison of the results of mass spec (MALDI-TOF) analysis of N-glycans of endogenous glycoproteins isolated from control tobacco and selected GnTIII-17 plant. See also Table 1.

m/z		Type	Wildtype endo	GnTIII-17 endo
1065		XM3	8	4
1079		FM3	3	0
1211		FXM3	30	4
1257		M5	2	3
1268		GNXM3	4	0
1414		GNFXM3	10	2
1419		M6	3	5
1471		GNbGNXM3		14
1471		GN2XM3	4	
1581		M7	3	4
1617		GN2FXM3	29	19
1617		GNbGNXM3		
1674		GN2bGNXM3	0	8
1743		M8	2	4
1820		GN2bGNFXM3	0	31
1905		M9	1	2
		TOTAL	99	100

Table 3. Overview N-glycans observed in control and transgenic GnTIII corn. Comparison of N-glycan structures (% of total) found on endogenous glycoproteins of control, untransformed corn and transgenic corn callus expressing GnTIII that could be annotated. Corresponding mass spectra obtained through MALDI-TOF analyses are given below and saccharides are indicated under column "name." Bisecting GlcNAc residues are depicted as bGN.

			Corn callus	
Structure abbreviation	**m/z**	**name**	**control**	**GnTIII**
XM3GN2	1065	a	1	5
FXM3GN2	1211	b	37	36
XM4GN2	1227	c	6	1
M5GN2	1257	d	1	1
GNFXM3GN2	1414	e	12	3
M6GN2	1419	f	5	4
GNXM4GN2	1430	g	3	
GN2XM3GN2 bGN2XM3GN2	1471	h		3
GNFXM4GN2	1576	i	1	
M7GN2	1581	j	1	1
GN2FXM3GN2 bGN2FXM3GN2	1617	k	16	19
M8GN2	1743	l	1	
bGN3FXM3GN2	1820	m		20
		Total	84	93

Table 4. Schematical overview N-glycans observed in control and transgenic GnTIII corn-2. Comparison of N-glycan structures (% of total) found on endogenous glycoproteins of control, untransformed corn and transgenic corn callus expressing GnTIII that could be annotated. Transgenic corn was selected using c-myc tag. Corresponding mass spectra obtained through MALDI-TOF analyses are given below and saccharides are indicated under column "name". Bisecting GlcNAc residues are depicted as bGN.

Structure abbreviation	**m/z**	**name**	**Corn callus**	
			control	**GnTIII-2**
XM3GN2	1065	a	1	
FXM3GN2	1211	b	37	9
XM4GN2	1227	c	6	
M5GN2	1257	d	1	
GNFXM3GN2	1414	e	12	
M6GN2	1419	f	5	
GNXM4GN2	1430	g	3	
GN2XM3GN2 bGN2XM3GN2	1471	h		
GNFXM4GN2	1576	i	1	
M7GN2	1581	j	1	
GN2FXM3GN2 bGN2FXM3GN2	1617	k	16	38
M8GN2	1743	l	1	
bGN3FXM3GN2	1820	m		54
		Total	84	101

Table 5. Positive test results for E-PHA binding.

Sample ID number	Rating for E-PHA Binding	Included in Pooled positive sample
1.	Unclear	
2.	Unclear	
3.	1	
4.	0	
5.	0	
6.	0	
7.	0	
8.	0	
9.	0	
10.	Unclear	
11.	Unclear	
12.	1	
13.	1	
14.	0	
15.	0	
16.	0	
17.	0	
18.	0	
19.	0	
20.	0	
21.	0	
22.	0	
23.	1	
24.	1	
25.	3	Yes
26.	3	Yes
27.	0	
28.	0	
29.	0	
30.	0	
31.	0	
32.	0	
33.	2	Yes
34.	1	
35.	0	
36.	0	
37.	Unclear	
38.	1	
39.	0	
40.	1	
41.	1	
42.	0	
43.	0	
44.	Unclear	
45.	0	
46.	0	

47.	0	
48.	2	Yes
49.	0	
50.	0	
51.	0	
52.	0	
53.	0	
54.	1	
55.	2	Yes
56.	2	Yes
57.	2	Yes
58.	1	
59.	2	Yes
60.	0	
61.	0	
62.	0	
63.	0	
64.	0	
65.	0	
66.	0	
67.	0	
68.	0	
69.	Negative control	

Table 6. Schematical overview N-glycans observed in control and transgenic GnTIII corn plants.

			Corn plant	
Structure abbreviation	**m/z**	**name**	**control**	**GnTIII**
XM3GN2	1065	a	4	14
FM3GN2	1079	b	2	
FXM3GN2	1211	c	59	30
XM4GN2	1227	d	3	12
GNFXM3GN2	1414	e	10	6
M6GN2	1419	f	2	
GN2FXM3GN2	1617	g	5	23
bGN2FXM3GN2				
M8GN2	1743	h	1	
bGN3FXM3GN2	1820	i		15
		Total	86	100

CLAIMS

What is Claimed is:

1. A plant host cell system, comprising a mammalian UDP-N-acetylglucosamine: β-D mannoside β(1,4)-N-acetylglucosaminyltransferase (GnTIII) enzyme.

2. The plant host system according to Claim 1, wherein said GnTIII is a human GnTIII.

3. The plant host system according to Claim 1, wherein said system is a portion of a plant.

4. The plant host system according to Claim 1, wherein said system is a portion of a plant selected from the group consisting of a cell, leaf, embryo, callus, stem, pericarp, protoplast, root, tuber, kernel, endosperm and embryo.

5. The plant host system according to Claim 1, wherein said system is a whole plant.

6. The plant host system according to Claim 1, further comprising a heterologous glycoprotein.

7. The plant host system according to Claim 5, wherein said heterologous glycoprotein protein comprises an antibody or fragment thereof.

8. The plant host system according to Claim 5, wherein said heterologous glycoprotein or functional fragment thereof comprises bisected oligosaccharides.

9. The plant host system according to Claim 5, wherein said heterologous glycoprotein comprises bisected glycans with galactose residues.

10. The plant host system according to Claim 1, wherein said plant is a tobacco plant.

11. The plant host system according to Claim 1, which further comprises a functional protein selected from the group consisting of a transporter and an enzyme providing N-glycan biosynthesis.

12. The plant host system according to Claim 11, wherein said enzyme is a (human) β-1,4 galactosyltransferase.

13. The plant host system according to Claim 11, which further comprises a heterologous glycoprotein, having an increased number of galactose residues.

14. A plant host system comprising a nucleic acid sequence encoding a mammalian GnTIII protein.

15. A plant host system comprising a vector comprising a nucleic acid sequence encoding a mammalian GnTIII protein.

16. The plant host system according to Claim 15, which further comprises a nucleic acid sequence encoding a functional protein selected from a group consisting of a transporter and an enzyme providing N-glycan biosynthesis.

17. A method comprising a) crossing a plant expressing a heterologous glycoprotein with a plant according to Claim 5, b) harvesting progeny from said crossing and c) selecting a desired progeny plant.

18. The method according to Claim 17, wherein said desired progeny plant expressing said heterologous glycoprotein protein having bisected oligosaccharides.

19. The method according to Claim 17, wherein said plant host system is a transgenic plant.

20. A method for obtaining a heterologous glycoprotein having bisected oligosaccharides comprising introducing a nucleic acid sequence encoding GnTIII that is normally not present in plant into a plant host system and a nucleic acid sequence encoding a heterologous glycoprotein and isolating said heterologous glycoprotein.

21. The method according to Claim 20, wherein said nucleic acid sequences are introduced into a plant cell and said plant cell is regenerated into a plant.

22. The method according to Claim 20, wherein said nucleic acid sequences are introduced into a plant host system by transforming said plant host system with a vector comprising a acid sequence encoding GnTIII that is normally not present in plant into a plant and a nucleic acid sequence encoding a heterologous glycopraxein.

23. The method according to Claim 20, wherein said nucleic acid sequences are introduced into a plant host system by transforming said plant host system with a vector comprising a nucleic acid sequence encoding GnTIII that is normally not present in plant into a plant and a nucleic acid sequence encoding a heterologous glycoprotein.

24. The method according to Claim 20, wherein said nucleic acid sequences are introduced into a plant host system by transforming said plant with a vector comprising a nucleic acid sequence

encoding GnTIII that is normally not present in plant into a plant host system and vector comprising a nucleic acid sequence encoding a heterologous glycoprotein.

25. A method for obtaining a heterologous glycoprotein having bisected oligosaccharides comprising cultivating the regenerated plant obtained in claim 21.

26. A method for obtaining a desired glycoprotein comprising a) cultivating the plant host system of Claim 6 and b) harvesting and fractionating said plant.

27. A plant obtainable by a method according to Claim 19.

28. A method for obtaining a plant host system comprising a) crossing a plant comprising a functional protein selected from a group consisting of a transporter or an enzyme providing N-glycan biosynthesis with a plant according to Claim 5, b) harvesting progeny from said crossing and c) selecting a desired progeny.

29. A transgenic plant obtained according to the method of Claim 28.

30. A method for increasing galactosylation of a heterologous glycoprotein expressed in a plant host system comprising a) introducing a nucleic acid sequence encoding GnTIII and a sequence selected from a group consisting of a transporter or an enzyme not normally present in a plant into said plant host system expressing said heterologous glycoprotein and b) isolating said glycoprotein.

31. A plant derived glycoprotein comprising bisected oligosaccharides.

32. Use of a plant host system according to Claims 1-16 to produce a desired glycoprotein or functional fragment thereof.

33. Use according to Claim 32 wherein a said glycoprotein or functional fragment thereof comprises bisected oligosaccharides.

34. A plant-derived glycoprotein or functional fragment thereof obtained by a method according to Claim 19.

35. Use of a glycoprotein or functional fragment thereof according to Claim 31 for the production of a pharmaceutical composition.

36. A composition comprising a glycoprotein or functional fragment thereof according to Claim 31.

37. An isolated hybrid protein comprising an active site of GnTIII and a transmembrane region of a protein, said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell.

38. The protein according to Claim 37, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is an enzyme.

39. The protein according to Claim 37, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is a glycosyltransferase.

40. The protein according to Claim 37, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is a glycosyltransferase selected from the group consisting of a mannosidaseI, mannosidaseII, GnTI, GnTII, Xy1T and FucT,

41. The protein according to Claim 37, wherein said protein residing in endoplasmic reticulum or Golgi apparatus of a eukaryotic cell is a plant protein.

42. An isolated nucleic acid sequence encoding the protein of Claim 31.

43. A vector comprising the isolated nucleic acid sequence of Claim 42.

44. A plant comprising the isolated nucleic acid sequence of Claim 42.

45. The plant according to Claim 44 which further comprises a nucleic acid sequence encoding a heterologous glycoprotein.

46. A method for providing a transgenic plant capable of expressing a heterologous glycoprotein with the capacity to extend an N-linked glycan with galactose comprising crossing a transgenic plant with a plant according to Claim 44, harvesting progeny from said crossing and selecting a desired progeny plant expressing said recombinant protein and expressing a functional (mammalian) enzyme involved in (mammalian) N-glycan biosynthesis that is normally not present in plants.

47. A method for providing a transgenic plant capable of expressing a heterologous glycoprotein with the capacity to extend an N-linked glycan with galactose comprising introducing the nucleic acid sequence of Claim 42 and a nucleic acid sequence encoding said heterologous glycoprotein.

48. A method, comprising:

a. providing: i) a plant cell, and ii) an expression vector comprising nucleic acid encoding a GNTIII enzyme; and
b. introducing said expression vector into said plant cell under conditions such that said enzyme is expressed.

49. The method of Claim 22, wherein said nucleic acid encoding a GNTIII comprises the nucleic acid sequence of SEQ ID NO:1.

50. A method, comprising:
a. providing: i) a plant cell, ii) a first expression vector comprising nucleic acid encoding a GNTIII enzyme, and iii) a second expression vector comprising nucleic acid encoding a heterologous glycoprotein; and
b. introducing said first and second expression vectors into said plant cell under conditions such that said hybrid enzyme and said heterologous protein are expressed.

51. The method of Claim 50, wherein said heterologous protein is an antibody or antibody fragment.

52. A method, comprising:
a) providing: i) a first plant comprising a first expression vector, said first vector comprising nucleic acid encoding a GNTIII enzyme, and ii) a second plant comprising a second expression vector, said second vector comprising nucleic acid encoding a heterologous protein; and
b) crossing said first plant and said second plant to produce progeny expressing said hybrid enzyme and said heterologous protein.

53. A plant, comprising first and second expression vectors, said first vector comprising nucleic acid encoding a GNTIII enzyme, said second vector comprising nucleic acid encoding a heterologous protein.

54. The plant of Claim 53, wherein said heterologous protein is an antibody or antibody fragment.

FIG. 1A

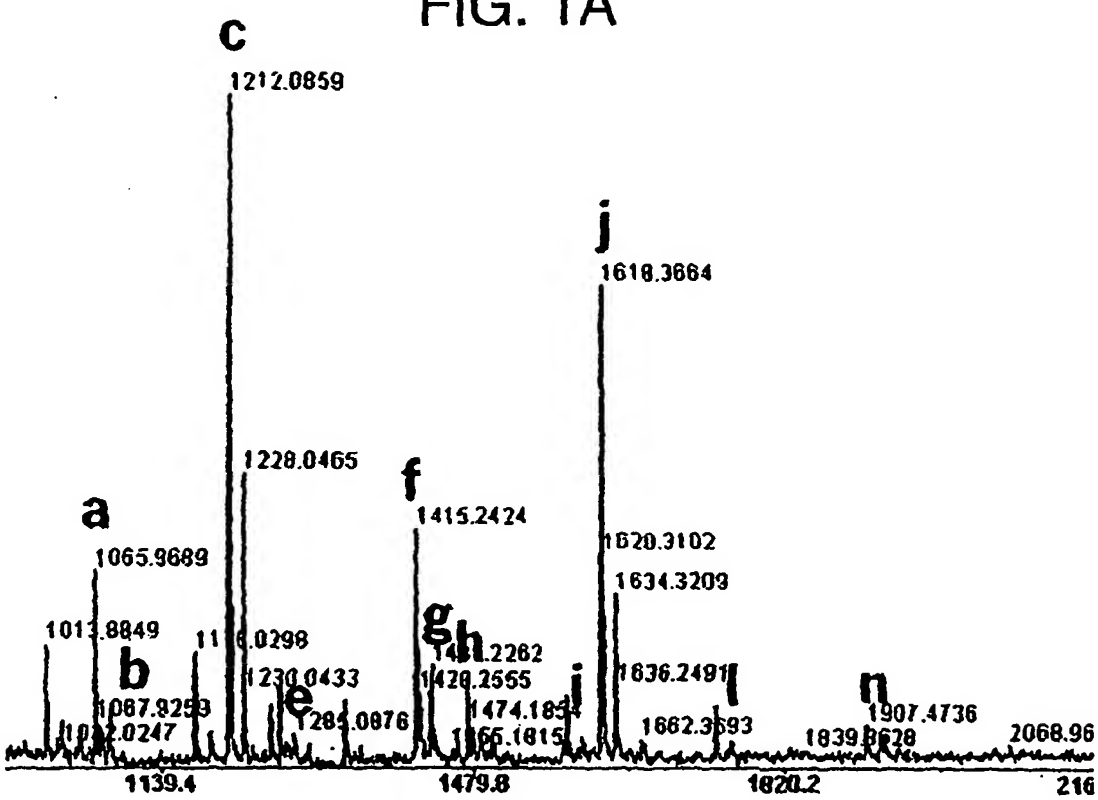
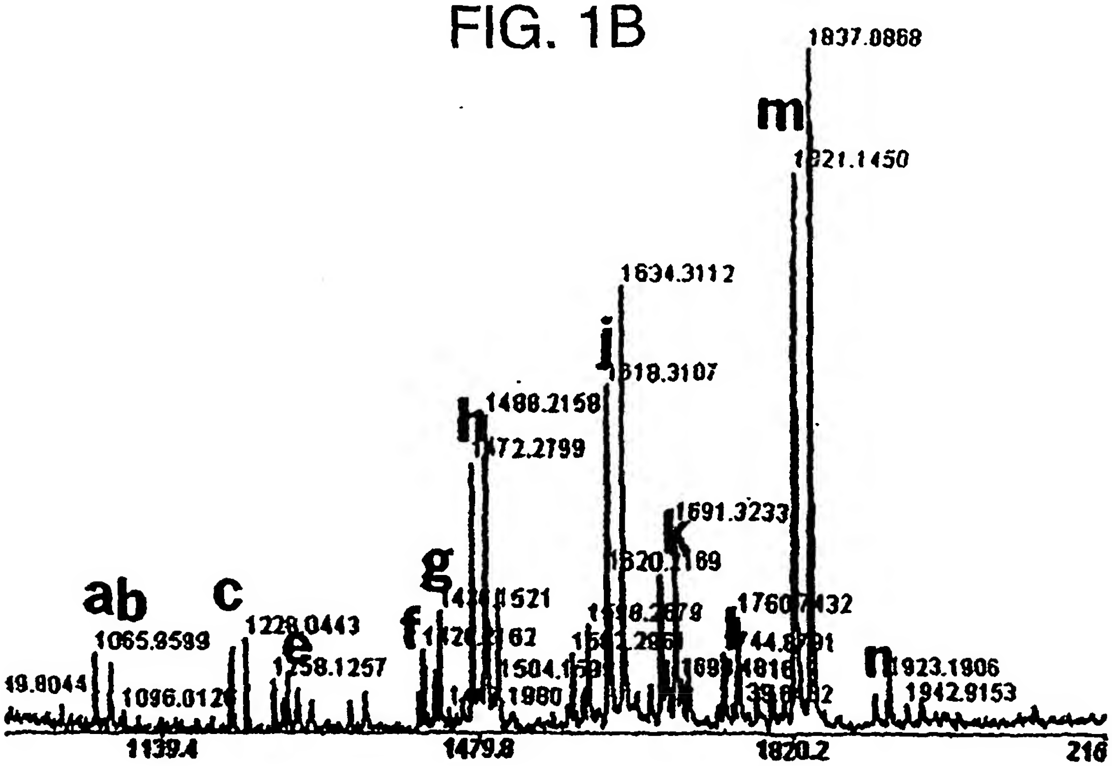
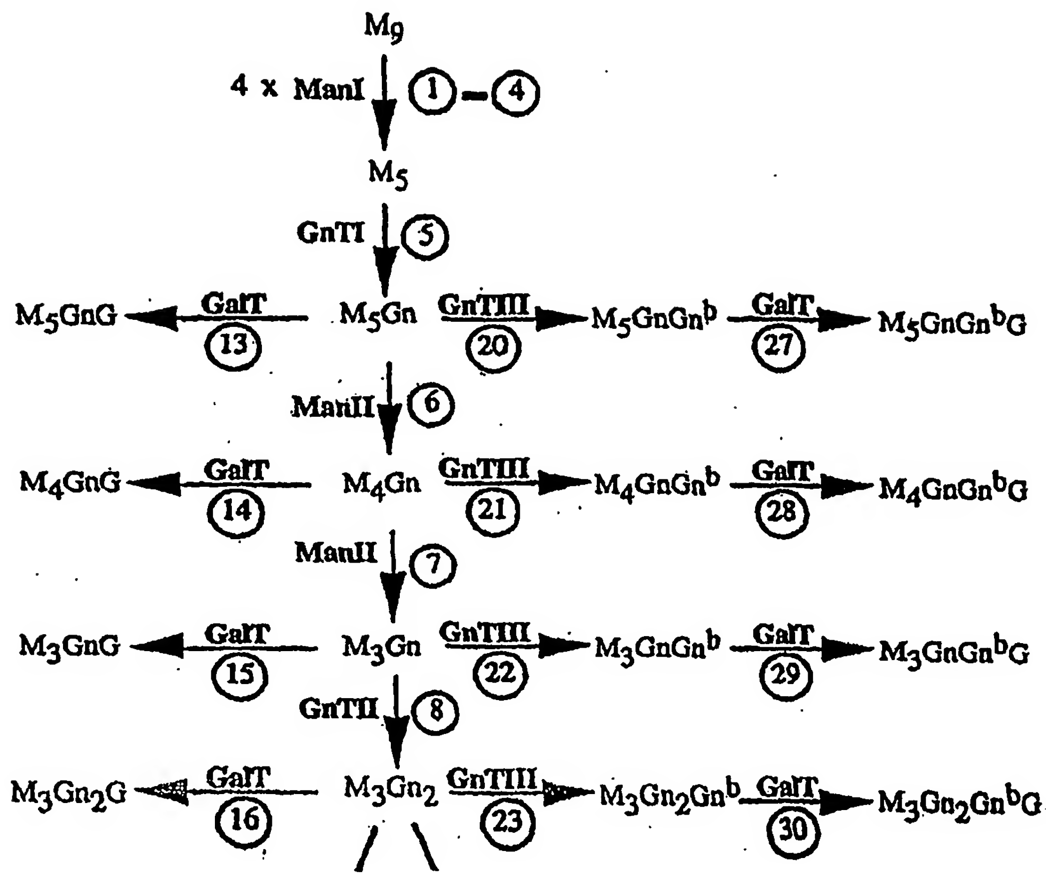


FIG. 1B




M9
4 x ManI
1 - 4
M5
GnTI
5
M5GnG
GalT
13
M5Gn
GnTIII
20
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GalT
27
M5GnGnbG
ManII
6
M4GnG
GalT
14
M4Gn
GnTIII
21
M4GnGnb
GalT
28
M4GnGnbG
ManII
7
M3GnG
GalT
15
M3Gn
GnTIII
22
M3GnGnb
GalT
29
M3GnGnbG
GnTII
8
M3Gn2G
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M3Gn2
GnTIII
23
M3Gn2Gnb
GalT
30
M3Gn2GnbG

FIG. 2

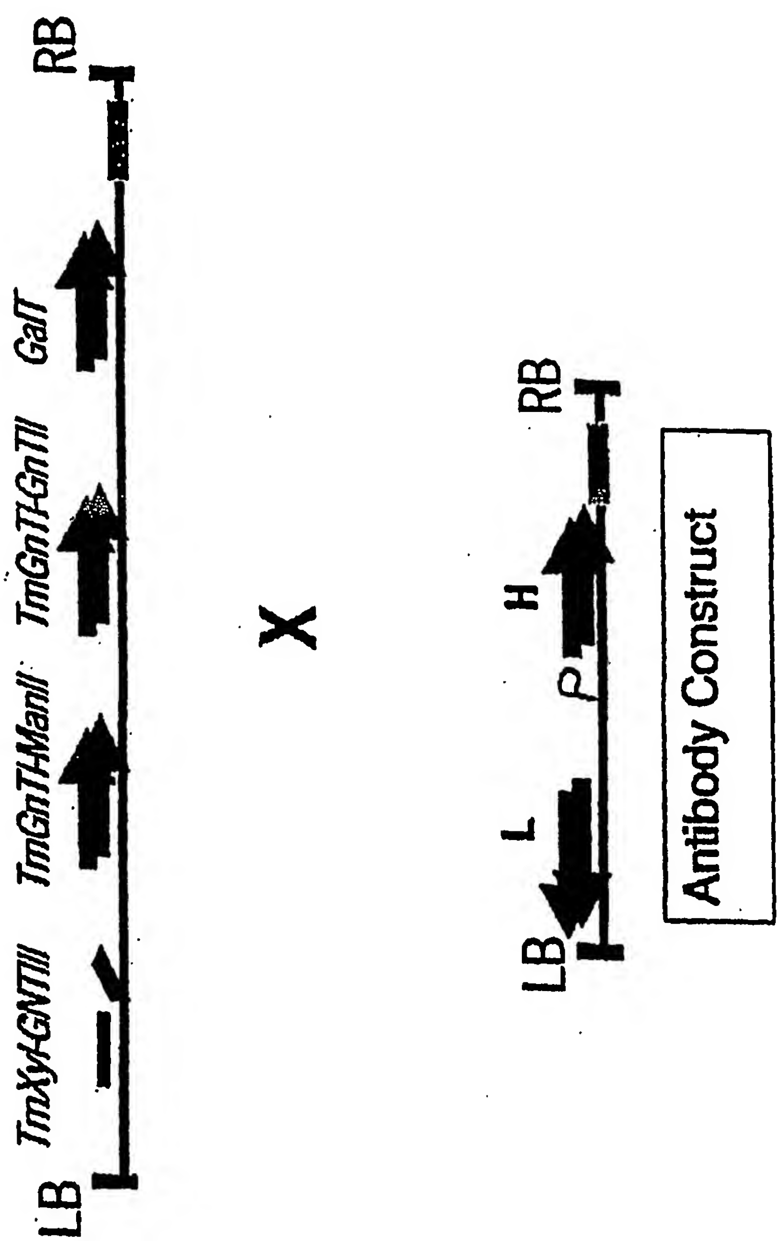
LB
TmXyl-GNTIII
TmGnTI-ManII
TmGnTI-GnTII
GalT
RB
X
LB
L
P
H
RB
Antibody Construct

FIG. 3

Nucleotide sequence (incl. myc-tag)

CCATGGTGATGAGACGCTACAAGCTCTTTCTCATGTTCTGTATGGCCGGCCTG
TGCCTCATCTCCTTCCTGCACTTCTTCAAGACCCTGTCCTATGTCACCTTCCCC
CGAGAACTGGCCTCCCTCAGCCCTAACCTGGTGTCCAGCTTTTTCTGGAACAA
TGCCCCGGTCACGCCCCAGGCCAGCCCCGAGCCAGGAGGCCCTGACCTGCTG
CGTACCCCACTCTACTCCCACTCGCCCCTGCTGCAGCCGCTGCCGCCCAGCAA
GGCGGCCGAGGAGCTCCACCGGGTGGACTTGGTGCTGCCCGAGGACACCACC
GAGTATTTCGTGCGCACCAAGGCCGGCGGCGTCTGCTTCAAACCCGGCACCA
AGATGCTGGAGAGGCCGCCCCCGGGACGGCCGGAGGAGAAGCCTGAGGGGG
CCAACGGCTCCTCGGCCCGGCGGCCACCCCGGTACCTCCTGAGCGCCCGGGA
GCGCACGGGGGGCCGAGGCGCCCGGCGCAAGTGGGTGGAGTGCGTGTGCCT
GCCCGGCTGGCACGGACCCAGCTGCGGCGTGCCCACTGTGGTGCAGTACTCC
AACCTGCCCACCAAGGAGCGGCTGGTGCCCAGGGAGGTGCCGCGCCGCGTCA
TCAACGCCATCAACGTCAACCACGAGTTCGACCTGCTGGACGTGCGCTTCCA
CGAGCTGGGCGACGTGGTGGACGCCTTTGTGGTGTGCGAGTCCAACTTCACG
GCTTATGGGGAGCCGCGGCCGCTCAAGTTCCGGGAGATGCTGACCAATGGCA
CCTTCGAGTACATCCGCCACAAGGTGCTCTATGTCTTCCTGGACCACTTCCCG
CCCGGCGGCCGGCAGGACGGCTGGATCGCCGACGACTACCTGCGCACCTTCC
TCACCCAGGACGGCGTCTCGCGGCTGCGCAACCTGCGGCCCGACGACGTCTT
CATCATTGACGATGCGGACGAGATCCCGGCCCGTGACGGCGTCCTTTTCCTCA
AGCTCTACGATGGCTGGACCGAGCCCTTCGCCTTCCACATGCGCAAGTCGCTC
TACGGCTTCTTCTGGAAGCAGCCGGGCACCCTGGAGGTGGTGTCAGGCTGCA
CGGTGGACATGCTGCAGGCAGTGTATGGGCTGGACGGCATCCGCCTGCGCCG
CCGCCAGTACTACACCATGCCCAACTTCAGACAGTATGAGAACCGCACCGGC
CACATCCTGGTGCAGTGGTCGCTGGGCAGCCCCCTGCACTTCGCCGGCTGGC
ACTGCTCCTGGTGCTTCACGCCCGAGGGCATCTACTTCAAGCTCGTGTCCGCC
CAGAATGGCGACTTCCCACGCTGGGGTGACTACGAGGACAAGCGGGACCTGA
ACTACATCCGCGGCCTGATCCGCACCGGGGGCTGGTTCGACGGCACGCAGCA
GGAGTACCCGCCTGCAGACCCCAGCGAGCACATGTATGCGCCCAAGTACCTG
CTGAAGAACTACGACCGGTTCCACTACCTGCTGGACAACCCCTACCAGGAGC
CCAGGAGCACGGCGGCGGGCGGGTGGCGCCACAGGGGTCCCGAGGGAAGGC
CGCCCGCCCGGGGCAAACTGGACGAGGCGGAAGTCGAACAAAAACTCATCT
CAGAAGAGGATCTGAATTAGGATCC

FIG. 4A

PROTEIN SEQUENCE

MVMRRYKLFL MFCMAGLCLI SFLHFFKTLS YVTFPRELAS LSPNLVSSFF
WNNAPVTPQA SPEPGGPDLL RTPLYSHSPL LQPLPPSKAA EELHRVDLVL
PEDTTEYFVR TKAGGVCFKP GTKMLERPPP GRPEEKPEGA NGSSARRPPR
YLLSARERTG GRGARRKWVE CVCLPGWHGP SCGVPTVVQY SNLPTKERLV
PREVPRRVIN AINVNHEFDL LDVRFHELGD VVDAFVVCES NFTAYGEPRP
LKFREMLTNG TFEYIRHKVL YVFLDHFPPG GRQDGWIADD YLRTFLTQDG
VSRLRNLRPD DVFIIDDADE IPARDGVLFL KLYDGWTEPF AFHMRKSLYG
FFWKQPGTLE VVSGCTVDML QAVYGLDGIR LRRRQYYTMP NFRQYENRTG
HILVQWSLGS PLHFAGWHCS WCFTPEGIYF KLVSAQNGDF PRWGDYEDKR
DLNYIRGLIR TGGWFDGTQQ EYPPADPSEH MYAPKYLLKN YDRFHYLLDN
PYQEPRSTAA GGWRHRGPEG RPPARGKLDE AEVEQKLISE EDLN

FIG. 4B

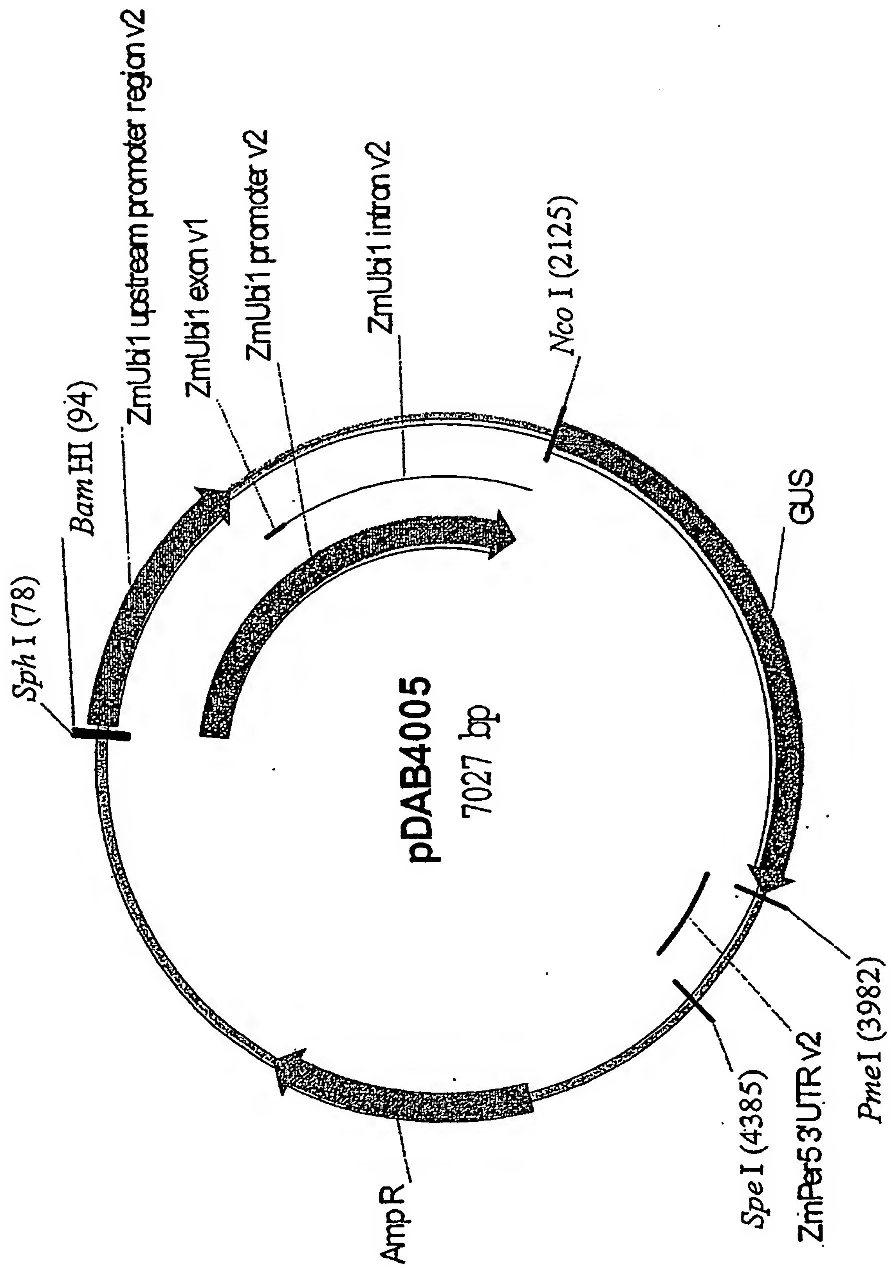
pDAB4005
7027 bp
Sph I (78)
Bam HI (94)
ZmUbi1 upstream promoter region v2
ZmUbi1 exon v1
ZmUbi1 promoter v2
ZmUbi1 intron v2
Nco I (2125)
GUS
Pme I (3982)
ZmPer5 3'UTR v2
Spe I (4385)
AmpR

FIG. 5A

Plasmid pDAB4005 (7027 bp)
Description: ZmUbi1promoter + intron/GUS/Per5 3'UTR

Nucleotide start	Nucleotide end	Sequence Feature
1	115	Linker sequence with multiple cloning site: CATGATTACGCCAAGCTAGCGGCCGCATTCCCGGGAAGCTA GGCCACCGTGGCCCGCCTGCAGGGGAAGCTTGCATGCCTGC AGATCCCCGGGGATCCTCTAGAGTCGACCTGCA
116	2105	Maize ubiquitin promoter + intron
1093	2105	Maize ubiquitin intron
2106	2125	Linker sequence: GGGTACCCCCGGGGTCGAC
2126	3967	GUS coding region
3968	3985	Linker sequence: TAATGAGCTCGTTTAAA
3986	4350	Maize peroxidase-5 3' UTR
4351	4405	Linker sequence: CGGCCGGCCTAGCTAGCCACGGTGGCCAGATCCACTAGTTC TAGAGCGGCCGCTT
4406	7027	Puc19
5006	5866	Ampicillin resistance gene

Sequence

```
  1 CATGATTACG CCAAGCTAGC GGCCGCATTC CCGGGAAGCT AGGCCACCGT
 51 GGCCCGCCTG CAGGGGAAGC TTGCATGCCT GCAGATCCCC GGGGATCCTC
101 TAGAGTCGAC CTGCAGTGCA GCGTGACCCG GTCGTGCCCC TCTCTAGAGA
```

FIG 5B

151 TAATGAGCAT TGCATGTCTA AGTTATAAAA AATTACCACA TATTTTTTTT
201 GTCACACTTG TTTGAAGTGC AGTTTATCTA TCTTTATACA TATATTTAAA
251 CTTTAATCTA CGAATAATAT AATCTATAGT ACTACAATAA TATCAGTGTT
301 TTAGAGAATC ATATAAATGA ACAGTTAGAC ATGGTCTAAA GGACAATTGA
351 GTATTTTGAC AACAGGACTC TACAGTTTTA TCTTTTTAGT GTGCATGTGT
401 TCTCCTTTTT TTTTGCAAAT AGCTTCACCT ATATAATACT TCATCCATTT
451 TATTAGTACA TCCATTTAGG GTTTAGGGTT AATGGTTTTT ATAGACTAAT
501 TTTTTTAGTA CATCTATTTT ATTCTATTTT AGCCTCTAAA TTAAGAAAAC
551 TAAAACTCTA TTTTAGTTTT TTTATTTAAT AATTTAGATA TAAAATAGAA
601 TAAAATAAAG TGACTAAAAA TTAAACAAAT ACCCTTTAAG AAATTAAAAA
651 AACTAAGGAA ACATTTTTCT TGTTTCGAGT AGATAATGCC AGCCTGTTAA
701 ACGCCGTCGA CGAGTCTAAC GGACACCAAC CAGCGAACCA GCAGCGTCGC
751 GTCGGGCCAA GCGAAGCAGA CGGCACGGCA TCTCTGTCGC TGCCTCTGGA
801 CCCCTCTCGA GAGTTCCGCT CCACCGTTGG ACTTGCTCCG CTGTCGGCAT
851 CCAGAAATTG CGTGGCGGAG CGGCAGACGT GAGCCGGCAC GGCAGGCGGC
901 CTCCTCCTCC TCTCACGGCA CGGCAGCTAC GGGGGATTCC TTTCCCACCG
951 CTCCTTCGCT TTCCCTTCCT CGCCCGCCGT AATAAATAGA CACCCCCTCC
1001 ACACCCTCTT TCCCCAACCT CGTGTTGTTC GGAGCGCACA CACACACAAC
1051 CAGATCTCCC CCAAATCCAC CCGTCGGCAC CTCCGCTTCA AGGTACGCCG
1101 CTCGTCCTCC CCCCCCCCCC CTCTCTACCT TCTCTAGATC GGCGTTCCGG
1151 TCCATGCATG GTTAGGGCCC GGTAGTTCTA CTTCTGTTCA TGTTTGTGTT
1201 AGATCCGTGT TTGTGTTAGA TCCGTGCTGC TAGCGTTCGT ACACGGATGC
1251 GACCTGTACG TCAGACACGT TCTGATTGCT AACTTGCCAG TGTTTCTCTT
1301 TGGGGAATCC TGGGATGGCT CTAGCCGTTC CGCAGACGGG ATCGATTTCA
1351 TGATTTTTTT TGTTTCGTTG CATAGGGTTT GGTTTGCCCT TTTCCTTTAT
1401 TTCAATATAT GCCGTGCACT TGTTTGTCGG GTCATCTTTT CATGCTTTTT
1451 TTTGTCTTGG TTGTGATGAT GTGGTCTGGT TGGGCGGTCG TTCTAGATCG
1501 GAGTAGAATT CTGTTTCAAA CTACCTGGTG GATTTATTAA TTTTGGATCT
1551 GTATGTGTGT GCCATACATA TTCATAGTTA CGAATTGAAG ATGATGGATG
1601 GAAATATCGA TCTAGGATAG GTATACATGT TGATGCGGGT TTTACTGATG
1651 CATATACAGA GATGCTTTTT GTTCGCTTGG TTGTGATGAT GTGGTGTGGT

FIG 5B CONT.

```
1701 TGGCGGTCG TTCATTCGTT CTAGATCGGA GTAGAATACT GTTTCAAACT
1751 ACCTGGTGTA TTTATTAATT TTGGAACTGT ATGTGTGTGT CATACATCTT
1801 CATAGTTACG AGTTTAAGAT GGATGGAAAT ATCGATCTAG GATAGGTATA
1851 CATGTTGATG TGGGTTTTAC TGATGCATAT ACATGATGGC ATATGCAGCA
1901 TCTATTCATA TGCTCTAACC TTGAGTACCT ATCTATTATA ATAAACAAGT
1951 ATGTTTTATA ATTATTTTGA TCTTGATATA CTTGGATGAT GGCATATGCA
2001 GCAGCTATAT GTGGATTTTT TTAGCCCTGC CTTCATACGC TATTTATTTG
2051 CTTGGTACTG TTTCTTTTGT CGATGCTCAC CCTGTTGTTT GGTGTTACTT
2101 CTGCAGGGTA CCCCCGGGGT CGACCATGGT AAGGGGCAGC CACCACCACC
2151 ACCACCACAT GGTCCGTCCT GTAGAAACCC CAACCCGTGA AATCAAAAAA
2201 CTCGACGGCC TGTGGGCATT CAGTCTGGAT CGCGAAAACT GTGGAATTGA
2251 TCAGCGTTGG TGGGAAAGCG CGTTACAAGA AAGCCGGGCA ATTGCTGTGC
2301 CAGGCAGTTT TAACGATCAG TTCGCCGATG CAGATATTCG TAATTATGCG
2351 GGCAACGTCT GGTATCAGCG CGAAGTCTTT ATACCGAAAG GTTGGGCAGG
2401 CCAGCGTATC GTGCTGCGTT TCGATGCGGT CACTCATTAC GGCAAAGTGT
2451 GGGTCAATAA TCAGGAAGTG ATGGAGCATC AGGGCGGCTA TACGCCATTT
2501 GAAGCCGATG TCACGCCGTA TGTTATTGCC GGGAAAAGTG TACGTATCAC
2551 CGTTTGTGTG AACAACGAAC TGAACTGGCA GACTATCCCG CCGGGAATGG
2601 TGATTACCGA CGAAAACGGC AAGAAAAAGC AGTCTTACTT CCATGATTTC
2651 TTTAACTATG CCGGAATCCA TCGCAGCGTA ATGCTCTACA CCACGCCGAA
2701 CACCTGGGTG GACGATATCA CCGTGGTGAC GCATGTCGCG CAAGACTGTA
2751 ACCACGCGTC TGTTGACTGG CAGGTGGTGG CCAATGGTGA TGTCAGCGTT
2801 GAACTGCGTG ATGCGGATCA ACAGGTGGTT GCAACTGGAC AAGGCACTAG
2851 CGGGACTTTG CAAGTGGTGA ATCCGCACCT CTGGCAACCG GGTGAAGGTT
2901 ATCTCTATGA ACTGTGCGTC ACAGCCAAAA GCCAGACAGA GTGTGATATC
2951 TACCCGCTTC GCGTCGGCAT CCGGTCAGTG GCAGTGAAGG GCGAACAGTT
3001 CCTGATTAAC CACAAACCGT TCTACTTTAC TGGCTTTGGT CGTCATGAAG
3051 ATGCGGACTT ACGTGGCAAA GGATTCGATA ACGTGCTGAT GGTGCACGAC
3101 CACGCATTAA TGGACTGGAT TGGGGCCAAC TCCTACCGTA CCTCGCATTA
3151 CCCTTACGCT GAAGAGATGC TCGACTGGGC AGATGAACAT GGCATCGTGG
3201 TGATTGATGA AACTGCTGCT GTCGGCTTTA ACCTCTCTTT AGGCATTGGT
```

FIG 5B CONT.

```
3251 TTCGAAGCGG GCAACAAGCC GAAAGAACTG TACAGCGAAG AGGCAGTCAA
3301 CGGGGAAACT CAGCAAGCGC ACTTACAGGC GATTAAAGAG CTGATAGCGC
3351 GTGACAAAAA CCACCCAAGC GTGGTGATGT GGAGTATTGC CAACGAACCG
3401 GATACCCGTC CGCAAGTGCA CGGGAATATT TCGCCACTGG CGGAAGCAAC
3451 GCGTAAACTC GACCCGACGC GTCCGATCAC CTGCGTCAAT GTAATGTTCT
3501 GCGACGCTCA CACCGATACC ATCAGCGATC TCTTTGATGT GCTGTGCCTG
3551 AACCGTTATT ACGGATGGTA TGTCCAAAGC GGCGATTTGG AAACGGCAGA
3601 GAAGGTACTG GAAAAAGAAC TTCTGGCCTG GCAGGAGAAA CTGCATCAGC
3651 CGATTATCAT CACCGAATAC GGCGTGGATA CGTTAGCCGG GCTGCACTCA
3701 ATGTACACCG ACATGTGGAG TGAAGAGTAT CAGTGTGCAT GGCTGGATAT
3751 GTATCACCGC GTCTTTGATC GCGTCAGCGC CGTCGTCGGT GAACAGGTAT
3801 GGAATTTCGC CGATTTTGCG ACCTCGCAAG GCATATTGCG CGTTGGCGGT
3851 AACAAGAAAG GGATCTTCAC TCGCGACCGC AAACCGAAGT CGGCGGCTTT
3901 TCTGCTGCAA AAACGCTGGA CTGGCATGAA CTTCGGTGAA AAACCGCAGC
3951 AGGGAGGCAA ACAATGATAA TGAGCTCGTT TAAACTGAGG GCACTGAAGT
4001 CGCTTGATGT GCTGAATTGT TTGTGATGTT GGTGGCGTAT TTTGTTTAAA
4051 TAAGTAAGCA TGGCTGTGAT TTTATCATAT GATCGATCTT TGGGGTTTTA
4101 TTTAACACAT TGTAAAATGT GTATCTATTA ATAACTCAAT GTATAAGATG
4151 TGTTCATTCT TCGGTTGCCA TAGATCTGCT TATTTGACCT GTGATGTTTT
4201 GACTCCAAAA ACCAAAATCA CAACTCAATA AACTCATGGA ATATGTCCAC
4251 CTGTTTCTTG AAGAGTTCAT CTACCATTCC AGTTGGCATT TATCAGTGTT
4301 GCAGCGGCGC TGTGCTTTGT AACATAACAA TTGTTACGGC ATATATCCAA
4351 CGGCCGGCCT AGCTAGCCAC GGTGGCCAGA TCCACTAGTT CTAGAGCGGC
4401 CGCTTAATTC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT
4451 GGCGTTACCC AACTTAATCG CCTTGCAGCA CATCCCCCTT TCGCCAGCTG
4501 GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGCA
4551 GCCTGAATGG CGAATGGCGC CTGATGCGGT ATTTTCTCCT TACGCATCTG
4601 TGCGGTATTT CACACCGCAT ATGGTGCACT CTCAGTACAA TCTGCTCTGA
4651 TGCCGCATAG TTAAGCCAGC CCCGACACCC GCCAACACCC GCTGACGCGC
4701 CCTGACGGGC TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC
4751 GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG
```

FIG 5B CONT.

```
4801 CGCGAGACGA AAGGGCCTCG TGATACGCCT ATTTTTATAG GTTAATGTCA
4851 TGATAATAAT GGTTTCTTAG ACGTCAGGTG GCACTTTTCG GGGAAATGTG
4901 CGCGGAACCC CTATTTGTTT ATTTTTCTAA ATACATTCAA ATATGTATCC
4951 GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAAGGA
5001 AGAGTATGAG TATTCAACAT TTCCGTGTCG CCCTTATTCC CTTTTTTGCG
5051 GCATTTTGCC TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA
5101 AGATGCTGAA GATCAGTTGG GTGCACGAGT GGGTTACATC GAACTGGATC
5151 TCAACAGCGG TAAGATCCTT GAGAGTTTTC GCCCCGAAGA ACGTTTTCCA
5201 ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGCGCGGTAT TATCCCGTAT
5251 TGACGCCGGG CAAGAGCAAC TCGGTCGCCG CATACACTAT TCTCAGAATG
5301 ACTTGGTTGA GTACTCACCA GTCACAGAAA AGCATCTTAC GGATGGCATG
5351 ACAGTAAGAG AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC
5401 GGCCAACTTA CTTCTGACAA CGATCGGAGG ACCGAAGGAG CTAACCGCTT
5451 TTTTGCACAA CATGGGGGAT CATGTAACTC GCCTTGATCG TTGGGAACCG
5501 GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA CGATGCCTGT
5551 AGCAATGGCA ACAACGTTGC GCAAACTATT AACTGGCGAA CTACTTACTC
5601 TAGCTTCCCG GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA
5651 GGACCACTTC TGCGCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA
5701 ATCTGGAGCC GGTGAGCGTG GGTCTCGCGG TATCATTGCA GCACTGGGGC
5751 CAGATGGTAA GCCCTCCCGT ATCGTAGTTA TCTACACGAC GGGGAGTCAG
5801 GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG GTGCCTCACT
5851 GATTAAGCAT TGGTAACTGT CAGACCAAGT TTACTCATAT ATACTTTAGA
5901 TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT
5951 TTTGATAATC TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG
6001 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTTT
6051 TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG
6101 GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC
6151 TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCCTTCTA GTGTAGCCGT
6201 AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT
6251 CTGCTAATCC TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT
6301 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG
```

FIG 5B CONT.

```
6351 GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC
6401 ACCGAACTGA GATACCTACA GCGTGAGCAT TGAGAAAGCG CCACGCTTCC
6451 CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG
6501 GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT
6551 CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC
6601 GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTAC
6651 GGTTCCTGGC CTTTTGCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA
6701 TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT GAGCTGATAC
6751 CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG
6801 CGGAAGAGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT
6851 CATTAATGCA GCTGGCACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA
6901 GCGCAACGCA ATTAATGTGA GTTAGCTCAC TCATTAGGCA CCCCAGGCTT
6951 TACACTTTAT GCTTCCGGCT CGTATGTTGT GTGGAATTGT GAGCGGATAA
7001 CAATTTCACA CAGGAAACAG CTATGAC
```

FIG 5B CONT.

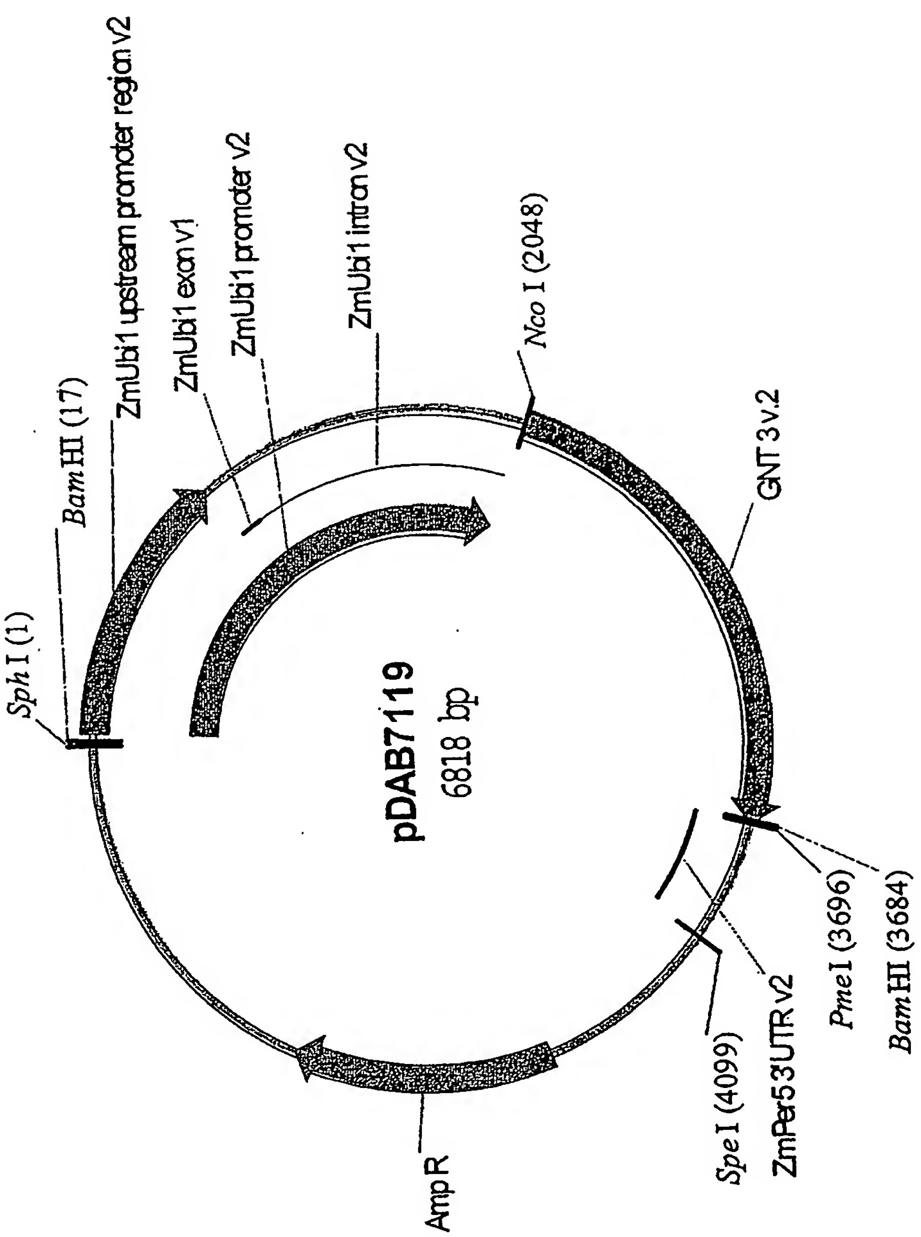
Sph I (1)
Bam HI (17)
ZmUbi1 upstream promoter region v2
ZmUbi1 exon v1
ZmUbi1 promoter v2
ZmUbi1 intron v2
Nco I (2048)
GNT 3 v.2
Pme I (3696)
Bam HI (3684)
Spe I (4099)
ZmPer5 3'UTR v2
Amp R
pDAB7119
6818 bp

FIG. 6A

pDAB7119 6818bp

Sequence	Feature
1-38 Linker	CCTGCAGATCCCCGGGGATCCTCTAGAGTCGACCTGCA
39-2028	Maize Ubiquitin 1 promoter
2029-2047 Linker	GGGTACCCCCGGGGTCGAC
2048-3695	GNT III v.2 plus TAGGTTT
2462	C to replace G as reported in original sequence
3696-3699 PmeI	AAAC
3700-4064	Maize Per 5 3'UTR
4065-6818	pUC19 backbone
4259-4264	TGCGCA FspI
4720-5580	Ampicillin Resistance gene
5282-5287	TGCGCA FspI

```
                              BamHI
                              ~~~~~~~
  1  CCTGCAGATC CCCGGGGATC CTCTAGAGTC GACCTGCAGT
     GCAGCGTGAC CCGGTCGTGC CCCTCTCTAG AGATAATGAG
     CATTGCATGT CTAAGTTATA
101  AAAAATTACC ACATATTTTT TTTGTCACAC TTGTTTGAAG
     TGCAGTTTAT CTATCTTTAT ACATATATTT AAACTTTAAT
     CTACGAATAA TATAATCTAT
201  AGTACTACAA TAATATCAGT GTTTTAGAGA ATCATATAAA
     TGAACAGTTA GACATGGTCT AAAGGACAAT TGAGTATTTT
     GACAACAGGA CTCTACAGTT
301  TTATCTTTTT AGTGTGCATG TGTTCTCCTT TTTTTTTGCA
     AATAGCTTCA CCTATATAAT ACTTCATCCA TTTTATTAGT
     ACATCCATTT AGGGTTTAGG
401  GTTAATGGTT TTTATAGACT AATTTTTTTA GTACATCTAT
     TTTATTCTAT TTTAGCCTCT AAATTAAGAA AACTAAAACT
     CTATTTTAGT TTTTTTATTT
501  AATAATTTAG ATATAAAATA GAATAAAATA AAGTGACTAA
     AAATTAAACA AATACCCTTT AAGAAATTAA AAAAACTAAG
     GAAACATTTT TCTTGTTTCG
601  AGTAGATAAT GCCAGCCTGT TAAACGCCGT CGACGAGTCT
     AACGGACACC AACCAGCGAA CCAGCAGCGT CGCGTCGGGC
     CAAGCGAAGC AGACGGCACG
701  GCATCTCTGT CGCTGCCTCT GGACCCCTCT CGAGAGTTCC
     GCTCCACCGT TGGACTTGCT CCGCTGTCGG CATCCAGAAA
     TTGCGTGGCG GAGCGGCAGA
801  CGTGAGCCGG CACGGCAGGC GGCCTCCTCC TCCTCTCACG
     GCACGGCAGC TACGGGGGAT TCCTTTCCCA CCGCTCCTTC
     GCTTTCCCTT CCTCGCCCGC
```

FIG 6B

901 CGTAATAAAT AGACACCCCC TCCACACCCT CTTTCCCCAA
CCTCGTGTTG TTCGGAGCGC ACACACACAC AACCAGATCT
CCCCCAAATC CACCCGTCGG
1001 CACCTCCGCT TCAAGGTACG CCGCTCGTCC TCCCCCCCCC
CCCCTCTCTA CCTTCTCTAG ATCGGCGTTC CGGTCCATGC
ATGGTTAGGG CCCGGTAGTT
1101 CTACTTCTGT TCATGTTTGT GTTAGATCCG TGTTTGTGTT
AGATCCGTGC TGCTAGCGTT CGTACACGGA TGCGACCTGT
ACGTCAGACA CGTTCTGATT
1201 GCTAACTTGC CAGTGTTTCT CTTTGGGGAA TCCTGGGATG
GCTCTAGCCG TTCCGCAGAC GGGATCGATT TCATGATTTT
TTTTGTTTCG TTGCATAGGG
1301 TTTGGTTTGC CCTTTTCCTT TATTTCAATA TATGCCGTGC
ACTTGTTTGT CGGGTCATCT TTTCATGCTT TTTTTTGTCT
TGGTTGTGAT GATGTGGTCT
1401 GGTTGGGCGG TCGTTCTAGA TCGGAGTAGA ATTCTGTTTC
AAACTACCTG GTGGATTTAT TAATTTTGGA TCTGTATGTG
TGTGCCATAC ATATTCATAG
1501 TTACGAATTG AAGATGATGG ATGGAAATAT CGATCTAGGA
TAGGTATACA TGTTGATGCG GGTTTTACTG ATGCATATAC
AGAGATGCTT TTTGTTCGCT
1601 TGGTTGTGAT GATGTGGTGT GGTTGGGCGG TCGTTCATTC
GTTCTAGATC GGAGTAGAAT ACTGTTTCAA ACTACCTGGT
GTATTTATTA ATTTTGGAAC
1701 TGTATGTGTG TGTCATACAT CTTCATAGTT ACGAGTTTAA
GATGGATGGA AATATCGATC TAGGATAGGT ATACATGTTG
ATGTGGGTTT TACTGATGCA
1801 TATACATGAT GGCATATGCA GCATCTATTC ATATGCTCTA
ACCTTGAGTA CCTATCTATT ATAATAAACA AGTATGTTTT
ATAATTATTT TGATCTTGAT
1901 ATACTTGGAT GATGGCATAT GCAGCAGCTA TATGTGGATT
TTTTTAGCCC TGCCTTCATA CGCTATTTAT TTGCTTGGTA
CTGTTTCTTT TGTCGATGCT

NcoI
FseI

\~~~~~~~
\~~~~~~~~~

2001 CACCCTGTTG TTTGGTGTTA CTTCTGCAGG GTACCCCCGG
GGTCGACCAT GGTGATGAGA CGCTACAAGC TCTTTCTCAT
GTTCTGTATG GCCGGCCTGT
2101 GCCTCATCTC CTTCCTGCAC TTCTTCAAGA CCCTGTCCTA
TGTCACCTTC CCCCGAGAAC TGGCCTCCCT CAGCCCTAAC
CTGGTGTCCA GCTTTTTCTG
2201 GAACAATGCC CCGGTCACGC CCCAGGCCAG CCCCGAGCCA
GGAGGCCCTG ACCTGCTGCG TACCCCACTC TACTCCCACT
CGCCCCTGCT GCAGCCGCTG

FIG 6B CONT.

```
                              SacI
     FspI
                                ~~~~~~
     ~~~~~~
2301 CCGCCCAGCA AGGCGGCCGA GGAGCTCCAC CGGGTGGACT
     TGGTGCTGCC CGAGGACACC ACCGAGTATT TCGTGCGCAC
     CAAGGCCGGC GGCGTCTGCT
2401 TCAAACCCGG CACCAAGATG CTGGAGAGGC CCCCCCCGGG
     ACGGCCGGAG GAGAAGCCTG AGGGGGCCAA CGGCTCCTCG
     GCCCGGCGGC CACCCCGGTA
2501 CCTCCTGAGC GCCCGGGAGC GCACGGGGGG CCGAGGCGCC
     CGGCGCAAGT GGGTGGAGTG CGTGTGCCTG CCCGGCTGGC
     ACGGACCCAG CTGCGGCGTG
2601 CCCACTGTGG TGCAGTACTC CAACCTGCCC ACCAAGGAGC
     GGCTGGTGCC CAGGGAGGTG CCGCGCCGCG TCATCAACGC
     CATCAACGTC AACCACGAGT

     NotI

     ~~~~~~~~
2701 TCGACCTGCT GGACGTGCGC TTCCACGAGC TGGGCGACGT
     GGTGGACGCC TTTGTGGTGT GCGAGTCCAA CTTCACGGCT
     TATGGGGAGC CGCGGCCGCT
2801 CAAGTTCCGG GAGATGCTGA CCAATGGCAC CTTCGAGTAC
     ATCCGCCACA AGGTGCTCTA TGTCTTCCTG GACCACTTCC
     CGCCCGGCGG CCGGCAGGAC
                                  FspI
     FspI
                                  ~~~~~~
     ~~~~~~~
2901 GGCTGGATCG CCGACGACTA CCTGCGCACC TTCCTCACCC
     AGGACGGCGT CTCGCGGCTG CGCAACCTGC GGCCCGACGA
     CGTCTTCATC ATTGACGATG

     FspI

     ~~~~~~~
3001 CGGACGAGAT CCCGGCCCGT GACGGCGTCC TTTTCCTCAA
     GCTCTACGAT GGCTGGACCG AGCCCTTCGC CTTCCACATG
     CGCAAGTCGC TCTACGGCTT
3101 CTTCTGGAAG CAGCCGGGCA CCCTGGAGGT GGTGTCAGGC
     TGCACGGTGG ACATGCTGCA GGCAGTGTAT GGGCTGGACG
     GCATCCGCCT GCGCCGCCGC
3201 CAGTACTACA CCATGCCCAA CTTCAGACAG TATGAGAACC
     GCACCGGCCA CATCCTGGTG CAGTGGTCGC TGGGCAGCCC
     CCTGCACTTC GCCGGCTGGC
```

FIG 6B CONT.

```
3301  ACTGCTCCTG GTGCTTCACG CCCGAGGGCA TCTACTTCAA
      GCTCGTGTCC GCCCAGAATG GCGACTTCCC ACGCTGGGGT
      GACTACGAGG ACAAGCGGGA
3401  CCTGAACTAC ATCCGCGGCC TGATCCGCAC CGGGGGCTGG
      TTCGACGGCA CGCAGCAGGA GTACCCGCCT GCAGACCCCA
      GCGAGCACAT GTATGCGCCC
3501  AAGTACCTGC TGAAGAACTA CGACCGGTTC CACTACCTGC
      TGGACAACCC CTACCAGGAG CCCAGGAGCA CGGCGGCGGG
      CGGGTGGCGC CACAGGGGTC

      BamHI       PmeI

      ~~~~~~    ~~~~~~~~

3601  CCGAGGGAAG GCCGCCCGCC CGGGGCAAAC TGGACGAGGC
      GGAAGTCGAA CAAAAACTCA TCTCAGAAGA GGATCTGAAT
      TAGGATCCTA GGTTTAAACT
3701  GAGGGCACTG AAGTCGCTTG ATGTGCTGAA TTGTTTGTGA
      TGTTGGTGGC GTATTTTGTT TAAATAAGTA AGCATGGCTG
      TGATTTTATC ATATGATCGA
3801  TCTTTGGGGT TTTATTTAAC ACATTGTAAA ATGTGTATCT
      ATTAATAACT CAATGTATAA GATGTGTTCA TTCTTCGGTT
      GCCATAGATC TGCTTATTTG
3901  ACCTGTGATG TTTTGACTCC AAAAACCAAA ATCACAACTC
      AATAAACTCA TGGAATATGT CCACCTGTTT CTTGAAGAGT
      TCATCTACCA TTCCAGTTGG

      FseI

      ~~~~~~~~~

4001  CATTTATCAG TGTTGCAGCG GCGCTGTGCT TTGTAACATA
      ACAATTGTTC ACGGCATATA TCCACGGCCG GCCTAGCTAG
      CCACGGTGGC CAGATCCACT
                 NotI
               ~~~~~~~~~
4101  AGTTCTAGAG CGGCCGCTTA ATTCACTGGC CGTCGTTTTA
      CAACGTCGTG ACTGGGAAAA CCCTGGCGTT ACCCAACTTA
      ATCGCCTTGC AGCACATCCC

      FspI

      ~~~~~~~

4201  CCTTTCGCCA GCTGGCGTAA TAGCGAAGAG GCCCGCACCG
      ATCGCCCTTC CCAACAGTTG CGCAGCCTGA ATGGCGAATG
      GCGCCTGATG CGGTATTTTC
4301  TCCTTACGCA TCTGTGCGGT ATTTCACACC GCATATGGTG
      CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC
      CAGCCCCGAC ACCCGCCAAC
```

FIG 6B CONT.

4401 ACCCGCTGAC GCGCCCTGAC GGGCTTGTCT GCTCCCGGCA
TCCGCTTACA GACAAGCTGT GACCGTCTCC GGGAGCTGCA
TGTGTCAGAG GTTTTCACCG
4501 TCATCACCGA AACGCGCGAG ACGAAAGGGC CTCGTGATAC
GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC
TTAGACGTCA GGTGGCACTT
4601 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT
CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA
CCCTGATAAA TGCTTCAATA
4701 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT
GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG
TTTTTGCTCA CCCAGAAACG
4801 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC
GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT
CCTTGAGAGT TTTCGCCCCG
4901 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT
ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG
CAACTCGGTC GCCGCATACA
5001 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA
GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT
GCAGTGCTGC CATAACCATG
5101 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG
GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG
GGATCATGTA ACTCGCCTTG

FspI

~~~~~~

5201 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA
CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG
TTGCGCAAAC TATTAACTGG
5301 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC
TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT
CGGCCCTTCC GGCTGGCTGG
5401 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC
GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC
CCGTATCGTA GTTATCTACA
5501 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA
GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA
CTGTCAGACC AAGTTTACTC
5601 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT
AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA
CCAAAATCCC TTAACGTGAG
5701 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA
AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG
CTGCTTGCAA ACAAAAAAAC

FIG 6B CONT.
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```
5801  CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT
      ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG
      CAGATACCAA ATACTGTCCT
5901  TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT
      GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC
      CAGTGGCTGC TGCCAGTGGC
6001  GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT
      TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC
      GTGCACACAG CCCAGCTTGG
6101  AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA
      GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG
      GACAGGTATC CGGTAAGCGG
6201  CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG
      GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC
      ACCTCTGACT TGAGCGTCGA
6301  TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA
      ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG
      CTGGCCTTTT GCTCACATGT
6401  TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT
      TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA
      ACGACCGAGC GCAGCGAGTC
6501  AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG
      CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC
      ACGACAGGTT TCCCGACTGG
6601  AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC
      TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC
      GGCTCGTATG TTGTGTGGAA

      NotI

      ~~~~~~~~~

6701  TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA
      CCATGATTAC GCCAAGCTAG CGGCCGCATT CCCGGGAAGC
      TAGGCCACCG TGGCCCGCCT
                 HindIII
                 ~~~~~~~
6801  GCAGGGGAAG CTTGCATG
```

FIG 6B CONT.

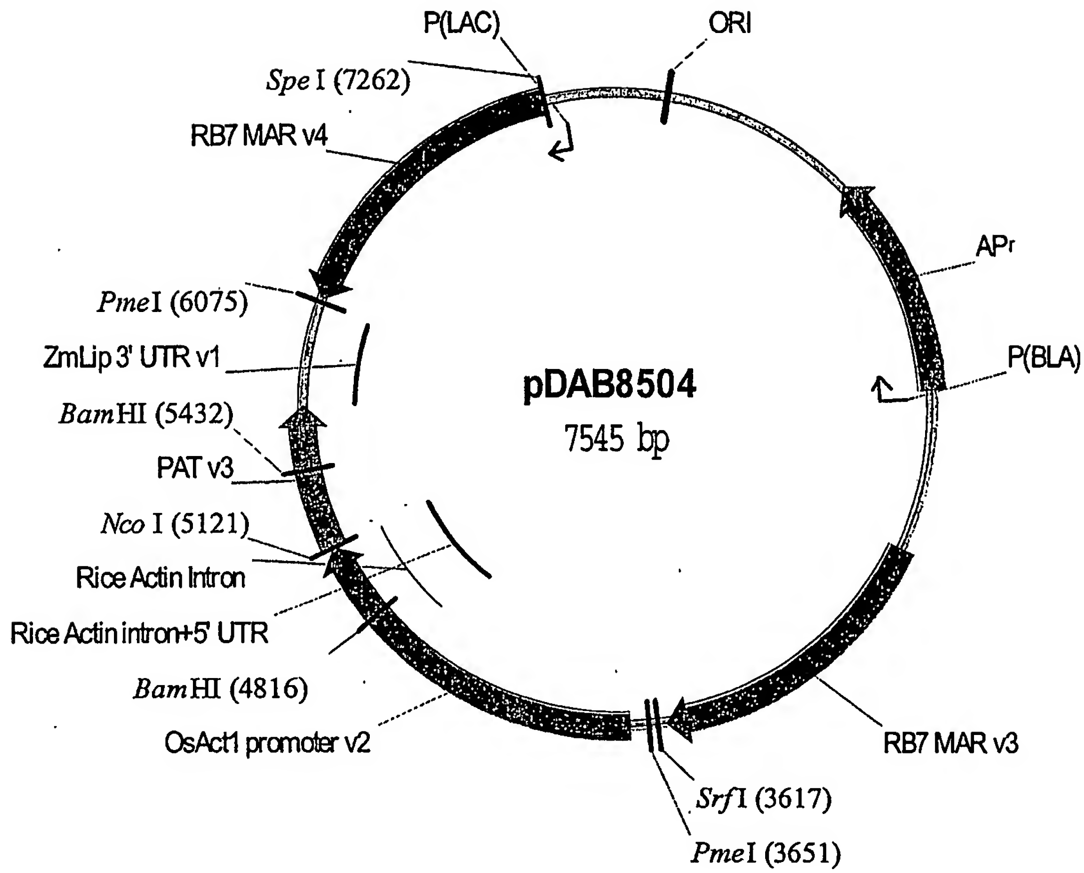
P(LAC)
ORI
Spe I (7262)
RB7 MAR v4
APr
*Pme*I (6075)
P(BLA)
ZmLip 3' UTR v1
pDAB8504
7545 bp
*Bam*HI (5432)
PAT v3
Nco I (5121)
Rice Actin Intron
Rice Actin intron+5' UTR
*Bam*HI (4816)
OsAct1 promoter v2
RB7 MAR v3
*Srf*I (3617)
*Pme*I (3651)

FIG. 7A

Plasmid pDAB8504 (7545 bp)
Description: Cloning vector with Rb7 MARs (inverted orientation) flanking a multiple cloning site and the rice actin/PAT/lipase selectable marker cassette

Nucleotide start	Nucleotide end	Sequence Feature
1	1166	Tobacco Rb7 MARs
1167	1304	Linker Sequence with multiple cloning site: TGGCCACCGCTTAATTAAGGCGCGCCATGCCCGGGCAAGCG GCCGCTTAATTAAATTTAAATGTTTAAACTAGGAAATCCAA GCTTGGGCTGCAGGTCAATCCCATTGCTTTTGAAGCAGCTC AACATTGATCTCTTT
1305	2701	Rice actin promoter with intron
2235	2696	Rice actin intron
2702	2703	CC
2704	3258	PAT gene (phosphinothricin acyl transferase)
3259	3272	Linker sequence: GGTACCCTGAGCTC
3273	3629	Maize lipase 3' UTR
3630	3670	Linker sequence: GAATTCATATTTCCTCCTGCAGGGTTTAAACTTGCCGTGGC
3671	4836	Tobacco Rb7 MAR (complementary)
4837	4857	Linker sequence: CGGCCCACTAGTCACCGGTGT
4858	5103	Puc19
5104	5130	Linker sequence: GCGCACGCTGCGCACGCTGCGCACGCT
5130	7523	Puc19
7524	7545	Linker sequence: ACACCGGTGTGATCATGGGCCG

Sequence

```
1     CGATTAAAAA TCTCAATTAT ATTTGGTCTA ATTTAGTTTG GTATTGAGTA
  51  AAACAAATTC GAACCAAACC AAAATATAAA TATATAGTTT TTATATATAT
 101  GCCTTTAAGA CTTTTTATAG AATTTTCTTT AAAAAATATC TAGAAATATT
 151  TGCGACTCTT CTGGCATGTA ATATTTCGTT AAATATGAAG TGCTCCATTT
 201  TTATTAACTT TAAATAATTG GTTGTACGAT CACTTTCTTA TCAAGTGTTA
 251  CTAAAATGCG TCAATCTCTT TGTTCTTCCA TATTCATATG TCAAAACCTA
 301  TCAAAATTCT TATATATCTT TTTCGAATTT GAAGTGAAAT TTCGATAATT
 351  TAAAATTAAA TAGAACATAT CATTATTTAG GTATCATATT GATTTTTATA
 401  CTTAATTACT AAATTTGGTT AACTTTGAAA GTGTACATCA ACGAAAAATT
 451  AGTCAAACGA CTAAAATAAA TAAATATCAT GTGTTATTAA GAAAATTCTC
 501  CTATAAGAAT ATTTTAATAG ATCATATGTT TGTAAAAAAA ATTAATTTTT
 551  ACTAACACAT ATATTTACTT ATCAAAAATT TGACAAAGTA AGATTAAAAT
 601  AATATTCATC TAACAAAAAA AAAACCAGAA AATGCTGAAA ACCCGGCAAA
 651  ACCGAACCAA TCCAAACCGA TATAGTTGGT TTGGTTTGAT TTTGATATAA
 701  ACCGAACCAA CTCGGTCCAT TTGCACCCCT AATCATAATA GCTTTAATAT
 751  TTCAAGATAT TATTAAGTTA ACGTTGTCAA TATCCTGGAA ATTTTGCAAA
 801  ATGAATCAAG CCTATATGGC TGTAATATGA ATTTAAAAGC AGCTCGATGT
 851  GGTGGTAATA TGTAATTTAC TTGATTCTAA AAAAATATCC CAAGTATTAA
```

FIG 7B

901 TAATTTCTGC TAGGAAGAAG GTTAGCTACG ATTTACAGCA AAGCCAGAAT
951 ACAATGAACC ATAAAGTGAT TGAAGCTCGA AATATACGAA GGAACAAATA
1001 TTTTTAAAAA AATACGCAAT GACTTGGAAC AAAAGAAAGT GATATATTTT
1051 TTGTTCTTAA ACAAGCATCC CCTCTAAAGA ATGGCAGTTT TCCTTTGCAT
1101 GTAACTATTA TGCTCCCTTC GTTACAAAAA TTTTGGACTA CTATTGGGAA
1151 CTTCTTCTGA AAATAGTGGC CACCGCTTAA TTAAGGCGCG CCATGCCCGG
1201 GCAAGCGGCC GCTTAATTAA ATTTAAATGT TTAAACTAGG AAATCCAAGC
1251 TTGGGCTGCA GGTCAATCCC ATTGCTTTTG AAGCAGCTCA ACATTGATCT
1301 CTTTCTCGAG GTCATTCATA TGCTTGAGAA GAGAGTCGGG ATAGTCCAAA
1351 ATAAAACAAA GGTAAGATTA CCTGGTCAAA AGTGAAAACA TCAGTTAAAA
1401 GGTGGTATAA AGTAAAATAT CGGTAATAAA AGGTGGCCCA AAGTGAAATT
1451 TACTCTTTTC TACTATTATA AAAATTGAGG ATGTTTTTGT CGGTACTTTG
1501 ATACGTCATT TTTGTATGAA TTGGTTTTTA AGTTTATTCG CTTTTGGAAA
1551 TGCATATCTG TATTTGAGTC GGGTTTTAAG TTCGTTTGCT TTTGTAAATA
1601 CAGAGGGATT TGTATAAGAA ATATCTTTAA AAAAACCCAT ATGCTAATTT
1651 GACATAATTT TTGAGAAAAA TATATATTCA GGCGAATTCT CACAATGAAC
1701 AATAATAAGA TTAAAATAGC TTTCCCCCGT TGCAGCGCAT GGGTATTTTT
1751 TCTAGTAAAA ATAAAAGATA AACTTAGACT CAAAACATTT ACAAAAACAA
1801 CCCCTAAAGT TCCTAAAGCC CAAAGTGCTA TCCACGATCC ATAGCAAGCC
1851 CAGCCCAACC CAACCCAACC CAACCCACCC CAGTCCAGCC AACTGGACAA
1901 TAGTCTCCAC ACCCCCCCAC TATCACCGTG AGTTGTCCGC ACGCACCGCA
1951 CGTCTCGCAG CCAAAAAAAA AAAAAGAAAG AAAAAAAAGA AAAAGAAAAA
2001 ACAGCAGGTG GGTCCGGGTC GTGGGGGCCG GAAACGCGAG GAGGATCGCG
2051 AGCCAGCGAC GAGGCCGGCC CTCCCTCCGC TTCCAAAGAA ACGCCCCCCA
2101 TCGCCACTAT ATACATACCC CCCCCTCTCC TCCCATCCCC CCAACCCTAC
2151 CACCACCACC ACCACCACCT CCACCTCCTC CCCCCTCGCT GCCGGACGAC
2201 GCCTCCCCCC TCCCCCTCCG CCGCCGCCGC GCCGGTAACC ACCCCGCCCC
2251 TCTCCTCTTT CTTTCTCCGT TTTTTTTTTC CGTCTCGGTC TCGATCTTTG
2301 GCCTTGGTAG TTTGGGTGGG CGAGAGGCGG CTTCGTGCGC GCCCAGATCG
2351 GTGCGCGGGA GGGGCGGGAT CTCGCGGCTG GGGCTCTCGC CGGCGTGGAT
2401 CCGGCCCGGA TCTCGCGGGG AATGGGGCTC TCGGATGTAG ATCTGCGATC
2451 CGCCGTTGTT GGGGGAGATG ATGGGGGGTT TAAAATTTCC GCCATGCTAA
2501 ACAAGATCAG GAAGAGGGGA AAAGGGCACT ATGGTTTATA TTTTTATATA
2551 TTTCTGCTGC TTCGTCAGGC TTAGATGTGC TAGATCTTTC TTTCTTCTTT
2601 TTGTGGGTAG AATTTGAATC CCTCAGCATT GTTCATCGGT AGTTTTTCTT
2651 TTCATGATTT GTGACAAATG CAGCCTCGTG CGGAGCTTTT TTGTAGGTAG
2701 ACCATGGCTT CTCCGGAGAG GAGACCAGTT GAGATTAGGC CAGCTACAGC
2751 AGCTGATATG GCCGCGGTTT GTGATATCGT TAACCATTAC ATTGAGACGT
2801 CTACAGTGAA CTTTAGGACA GAGCCACAAA CACCACAAGA GTGGATTGAT
2851 GATCTAGAGA GGTTGCAAGA TAGATACCCT TGGTTGGTTG CTGAGGTTGA
2901 GGGTGTTGTG GCTGGTATTG CTTACGCTGG GCCCTGGAAG GCTAGGAACG
2951 CTTACGATTG GACAGTTGAG AGTACTGTTT ACGTGTCACA TAGGCATCAA
3001 AGGTTGGGCC TAGGATCCAC ATTGTACACA CATTTGCTTA AGTCTATGGA
3051 GGCGCAAGGT TTTAAGTCTG TGGTTGCTGT TATAGGCCTT CCAAACGATC
3101 CATCTGTTAG GTTGCATGAG GCTTTGGGAT ACACAGCCCG GGGTACATTG
3151 CGCGCAGCTG GATACAAGCA TGGTGGATGG CATGATGTTG GTTTTTGGCA
3201 AAGGGATTTT GAGTTGCCAG CTCCTCCAAG GCCAGTTAGG CCAGTTACCC
3251 AGATCTGAGG TACCCTGAGC TCGGTCGCAG CGTGTGCGTG TCCGTCGTAC
3301 GTTCTGGCCG GCCGGGCCTT GGGCGCGCGA TCAGAAGCGT TGCGTTGGCG
3351 TGTGTGTGCT TCTGGTTTGC TTTAATTTTA CCAAGTTTGT TTCAAGGTGG
3401 ATCGCGTGGT CAAGGCCCGT GTGCTTTAAA GACCCACCGG CACTGGCAGT
3451 GAGTGTTGCT GCTTGTGTAG GCTTTGGTAC GTATGGGCTT TATTTGCTTC

FIG 7B CONT.

3501	TGGATGTTGT	GTACTACTTG	GGTTTGTTGA	ATTATTATGA	GCAGTTGCGT
3551	ATTGTAATTC	AGCTGGGCTA	CCTGGACATT	GTTATGTATT	AATAAATGCT
3601	TTGCTTTCTT	CTAAAGATCT	TTAAGTGCTG	AATTCATATT	TCCTCCTGCA
3651	GGGTTTAAAC	TTGCCGTGGC	CTATTTTCAG	AAGAAGTTCC	CAATAGTAGT
3701	CCAAAATTTT	TGTAACGAAG	GGAGCATAAT	AGTTACATGC	AAAGGAAAAC
3751	TGCCATTCTT	TAGAGGGGAT	GCTTGTTTAA	GAACAAAAAA	TATATCACTT
3801	TCTTTTGTTC	CAAGTCATTG	CGTATTTTTT	TAAAAATATT	TGTTCCTTCG
3851	TATATTTCGA	GCTTCAATCA	CTTTATGGTT	CTTTGTATTC	TGGCTTTGCT
3901	GTAAATCGTA	GCTAACCTTC	TTCCTAGCAG	AAATTATTAA	TACTTGGGAT
3951	ATTTTTTTAG	AATCAAGTAA	ATTACATATT	ACCACCACAT	CGAGCTGCTT
4001	TTAAATTCAT	ATTACAGCCA	TATAGGCTTG	ATTCATTTTG	CAAAATTTCC
4051	AGGATATTGA	CAACGTTAAC	TTAATAATAT	CTTGAAATAT	TAAAGCTATT
4101	ATGATTAGGG	GTGCAAATGG	ACCGAGTTGG	TTCGGTTTAT	ATCAAAATCA
4151	AACCAAACCA	ACTATATCGG	TTTGGATTGG	TTCGGTTTTG	CCGGGTTTTC
4201	AGCATTTTCT	GGTTTTTTTT	TTGTTAGATG	AATATTATTT	TAATCTTACT
4251	TTGTCAAATT	TTTGATAAGT	AAATATATGT	GTTAGTAAAA	ATTAATTTTT
4301	TTTACAAACA	TATGATCTAT	TAAAATATTC	TTATAGGAGA	ATTTTCTTAA
4351	TAACACATGA	TATTTATTTA	TTTTAGTCGT	TTGACTAATT	TTTCGTTGAT
4401	GTACACTTTC	AAAGTTAACC	AAATTTAGTA	ATTAAGTATA	AAAATCAATA
4451	TGATACCTAA	ATAATGATAT	GTTCTATTTA	ATTTTAAATT	ATCGAAATTT
4501	CACTTCAAAT	TCGAAAAAGA	TATATAAGAA	TTTTGATAGA	TTTTGACATA
4551	TGAATATGGA	AGAACAAAGA	GATTGACGCA	TTTTAGTAAC	ACTTGATAAG
4601	AAAGTGATCG	TACAACCAAT	TATTTAAAGT	TAATAAAAAT	GGAGCACTTC
4651	ATATTTAACG	AAATATTACA	TGCCAGAAGA	GTCGCAAATA	TTTCTAGATA
4701	TTTTTTAAAG	AAAATTCTAT	AAAAAGTCTT	AAAGGCATAT	ATATAAAAAC
4751	TATATATTTA	TATTTTGGTT	TGGTTCGAAT	TTGTTTTACT	CAATACCAAA
4801	CTAAATTAGA	CCAAATATAA	TTGGGATTTT	TAATCGCGGC	CCACTAGTCA
4851	CCGGTGTAGC	TTGGCGTAAT	CATGGTCATA	GCTGTTTCCT	GTGTGAAATT
4901	GTTATCCGCT	CACAATTCCA	CACAACATAC	GAGCCGGAAG	CATAAAGTGT
4951	AAAGCCTGGG	GTGCCTAATG	AGTGAGCTAA	CTCACATTAA	TTGCGTTGCG
5001	CTCACTGCCC	GCTTTCCAGT	CGGGAAACCT	GTCGTGCCAG	CTGCATTAAT
5051	GAATCGGCCA	ACGCGCGGGG	AGAGGCGGTT	TGCGTATTGG	GCGCTCTTCC
5101	GCTGCGCACG	CTGCGCACGC	TGCGCACGCT	TCCTCGCTCA	CTGACTCGCT
5151	GCGCTCGGTC	GTTCGGCTGC	GGCGAGCGGT	ATCAGCTCAC	TCAAAGGCGG
5201	TAATACGGTT	ATCCACAGAA	TCAGGGGATA	ACGCAGGAAA	GAACATGTGA
5251	GCAAAAGGCC	AGCAAAAGGC	CAGGAACCGT	AAAAAGGCCG	CGTTGCTGGC
5301	GTTTTTCCAT	AGGCTCCGCC	CCCCTGACGA	GCATCACAAA	AATCGACGCT
5351	CAAGTCAGAG	GTGGCGAAAC	CCGACAGGAC	TATAAAGATA	CCAGGCGTTT
5401	CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCCT	GTTCCGACCC	TGCCGCTTAC
5451	CGGATACCTG	TCCGCCTTTC	TCCCTTCGGG	AAGCGTGGCG	CTTTCTCATA
5501	GCTCACGCTG	TAGGTATCTC	AGTTCGGTGT	AGGTCGTTCG	CTCCAAGCTG
5551	GGCTGTGTGC	ACGAACCCCC	CGTTCAGCCC	GACCGCTGCG	CCTTATCCGG
5601	TAACTATCGT	CTTGAGTCCA	ACCCGGTAAG	ACACGACTTA	TCGCCACTGG
5651	CAGCAGCCAC	TGGTAACAGG	ATTAGCAGAG	CGAGGTATGT	AGGCGGTGCT
5701	ACAGAGTTCT	TGAAGTGGTG	GCCTAACTAC	GGCTACACTA	GAAGGACAGT
5751	ATTTGGTATC	TGCGCTCTGC	TGAAGCCAGT	TACCTTCGGA	AAAAGAGTTG
5801	GTAGCTCTTG	ATCCGGCAAA	CAAACCACCG	CTGGTAGCGG	TGGTTTTTTT
5851	GTTTGCAAGC	AGCAGATTAC	GCGCAGAAAA	AAAGGATCTC	AAGAAGATCC
5901	TTTGATCTTT	TCTACGGGGT	CTGACGCTCA	GTGGAACGAA	AACTCACGTT
5951	AAGGGATTTT	GGTCATGAGA	TTATCAAAAA	GGATCTTCAC	CTAGATCCTT
6001	TTAAATTAAA	AATGAAGTTT	TAAATCAATC	TAAAGTATAT	ATGAGTAAAC
6051	TTGGTCTGAC	AGTTACCAAT	GCTTAATCAG	TGAGGCACCT	ATCTCAGCGA

FIG 7B CONT.

```
6101  TCTGTCTATT TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA
6151  ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG CAATGATACC
6201  GCGAGACCCA CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAGCCAG
6251  CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC CGCCTCCATC
6301  CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA
6351  TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT
6401  CGTCGTTTGG TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA
6451  GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC
6501  TCCGATCGTT GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA
6551  TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT
6601  TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG
6651  GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC
6701  ATAGCAGAAC TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA
6751  AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA TGTAACCCAC
6801  TCGTGCACCC AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG
6851  GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG
6901  ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG
6951  CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT
7001  AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA
7051  CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG
7101  GCGTATCACG AGGCCCTTTC GTCTCGCGCG TTTCGGTGAT GACGGTGAAA
7151  ACCTCTGACA CATGCAGCTC CCGGAGACGG TCACAGCTTG TCTGTAAGCG
7201  GATGCCGGGA GCAGACAAGC CCGTCAGGGC GCGTCAGCGG GTGTTGGCGG
7251  GTGTCGGGGC TGGCTTAACT ATGCGGCATC AGAGCAGATT GTACTGAGAG
7301  TGCACCATAT GCGGTGTGAA ATACCGCACA GATGCGTAAG GAGAAAATAC
7351  CGCATCAGGC GCCATTCGCC ATTCAGGCTG CGCAACTGTT GGGAAGGGCG
7401  ATCGGTGCGG GCCTCTTCGC TATTACGCCA GCTGGCGAAA GGGGGATGTG
7451  CTGCAAGGCG ATTAAGTTGG GTAACGCCAG GGTTTTCCCA GTCACGACGT
7501  TGTAAAACGA CGGCCAGTGA ATTACACCGG TGTGATCATG GGCCG
```

FIG 7B CONT.

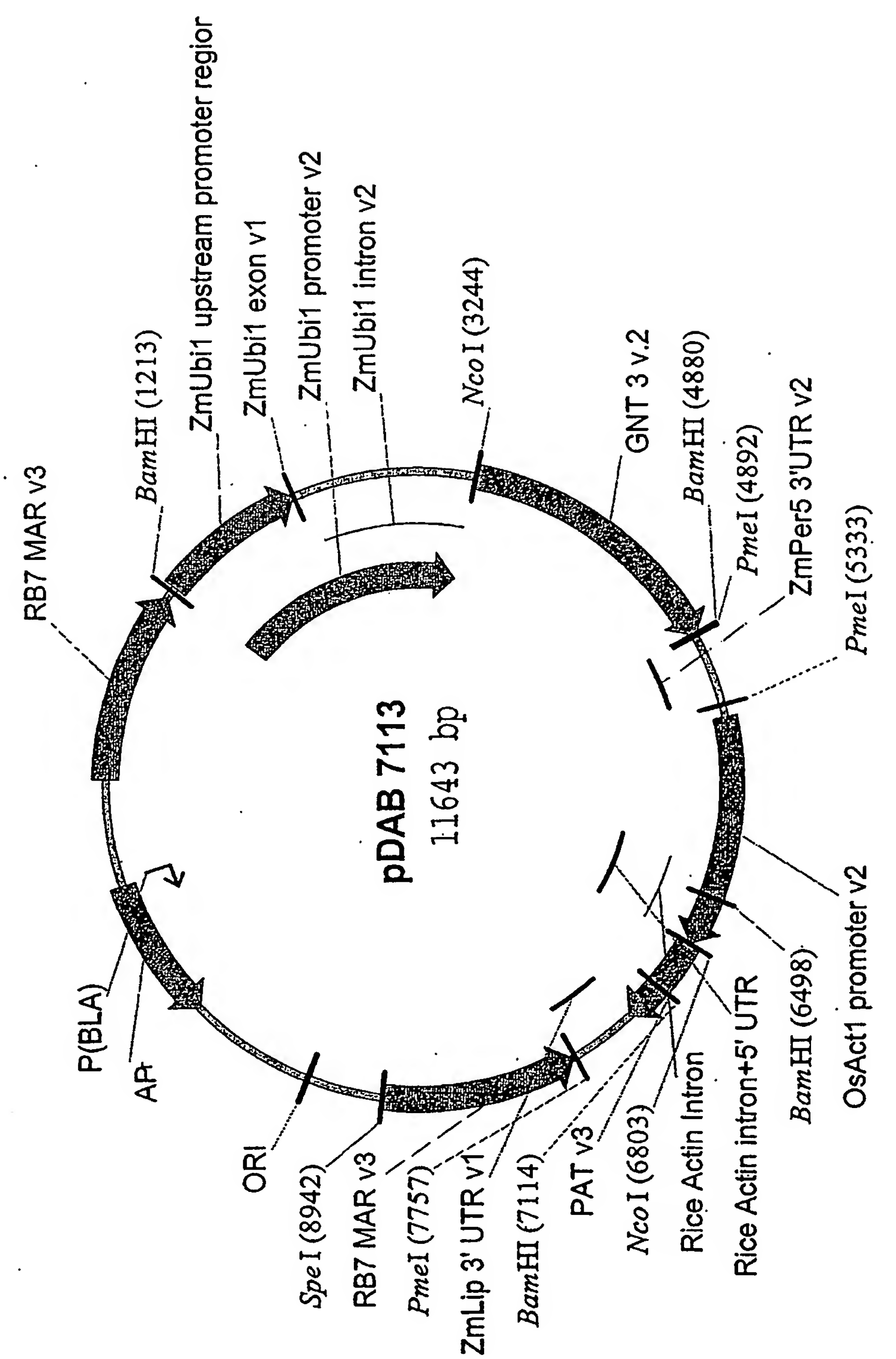
pDAB 7113
11643 bp
RB7 MAR v3
BamHI (1213)
ZmUbi1 upstream promoter regior
ZmUbi1 exon v1
ZmUbi1 promoter v2
ZmUbi1 intron v2
NcoI (3244)
GNT 3 v.2
BamHI (4880)
PmeI (4892)
ZmPer5 3'UTR v2
PmeI (5333)
OsAct1 promoter v2
BamHI (6498)
Rice Actin intron+5' UTR
Rice Actin Intron
NcoI (6803)
PAT v3
BamHI (7114)
ZmLip 3' UTR v1
PmeI (7757)
RB7 MAR v3
SpeI (8942)
ORI
AP
P(BLA)

FIG. 8A

pDAB7113 11643bp

Sequence	Feature
1-1164bp	RB7 MAR v3
1165-1233 Linker	TGGCCACCGCTTAATTAAGGCGCGCCATGCCCCCTGCAG ATCCCCGGGGATCCTCTAGAGTCGACCTGC
1234-3224	Maize Ubiquitin 1 promoter
3224-4891	GNT III v.2
3627	C to replace G as reported in original sequence
4892-4895 Linker	TAGGTTT
4896-5260	Maize Per5 3'UTR v2
5261-5404 multiple cloning sites	CGGCCGGCCTAGCTAGCCACGGTGGCCAGATCCACTAGG GGCAAGCGGCCGCTTAATTAAATTTAAATGTTTAAACTA GGAAATCCAAGCTTGGGCTGCAGGTCAATCCCATTGCTT TTGAAGCAGCTCAACATTGATCTCTTT
5405-6802	Rice Actin 1 Promoter v2
6803-7358	PAT v3
7359-7372 Linker	GGTACCCTGAGCTC
7373-7729	Maize Lipase 3' UTR v1
7730-7770 Linker	GAATTCATATTTCCTCCTGCAGGGTTTAAACTTGCCGTG GC
7771-8934	RB7 MAR v3
8935-11643	PUC19
9201-9225	3 FspI sites (TGCGCAA) with CG in between sites
10164-11021	Ampicillin resistance gene
10454-10459	TGCGCAA FspI
11477-11482	TGCGCAA FspI

```
  1 CGATTAAAAA CCCAATTATA TTTGGTCTAA TTTAGTTTGG
    TATTGAGTAA AACAAATTCG AACCAAACCA AAATATAAAT
    ATATAGTTTT TATATATATG
101 CCTTTAAGAC TTTTTATAGA ATTTTCTTTA AAAAATATCT
    AGAAATATTT GCGACTCTTC TGGCATGTAA TATTTCGTTA
    AATATGAAGT GCTCCATTTT
201 TATTAACTTT AAATAATTGG TTGTACGATC ACTTTCTTAT
    CAAGTGTTAC TAAAATGCGT CAATCTCTTT GTTCTTCCAT
    ATTCATATGT CAAAATCTAT
301 CAAAATTCTT ATATATCTTT TTCGAATTTG AAGTGAAATT
    TCGATAATTT AAAATTAAAT AGAACATATC ATTATTTAGG
    TATCATATTG ATTTTTATAC
```

FIG 8B

```
 401  TTAATTACTA AATTTGGTTA ACTTTGAAAG TGTACATCAA
      CGAAAAATTA GTCAAACGAC TAAAATAAAT AAATATCATG
      TGTTATTAAG AAAATTCTCC
 501  TATAAGAATA TTTTAATAGA TCATATGTTT GTAAAAAAAA
      TTAATTTTTA CTAACACATA TATTTACTTA TCAAAAATTT
      GACAAAGTAA GATTAAAATA
 601  ATATTCATCT AACAAAAAAA AAACCAGAAA ATGCTGAAAA
      CCCGGCAAAA CCGAACCAAT CCAAACCGAT ATAGTTGGTT
      TGGTTTGATT TTGATATAAA
 701  CCGAACCAAC TCGGTCCATT TGCACCCCTA ATCATAATAG
      CTTTAATATT TCAAGATATT ATTAAGTTAA CGTTGTCAAT
      ATCCTGGAAA TTTTGCAAAA
 801  TGAATCAAGC CTATATGGCT GTAATATGAA TTTAAAAGCA
      GCTCGATGTG GTGGTAATAT GTAATTTACT TGATTCTAAA
      AAAATATCCC AAGTATTAAT
 901  AATTTCTGCT AGGAAGAAGG TTAGCTACGA TTTACAGCAA
      AGCCAGAATA CAAAGAACCA TAAAGTGATT GAAGCTCGAA
      ATATACGAAG GAACAAATAT
1001  TTTTAAAAAA ATACGCAATG ACTTGGAACA AAAGAAAGTG
      ATATATTTTT TGTTCTTAAA CAAGCATCCC CTCTAAAGAA
      TGGCAGTTTT CCTTTGCATG

      PacI

      ~~~~~~~~~

      AscI

      ~~~~~~~~~
1101  TAACTATTAT GCTCCCTTCG TTACAAAAAT TTTGGACTAC
      TATTGGGAAT TCTTCTGAAA ATAGTGGCCA CCGCTTAATT
      AAGGCGCGCC ATGCCCCCTG
                   BamHI
                   ~~~~~~
1201  CAGATCCCCG GGGATCCTCT AGAGTCGACC TGCAGTGCAG
      CGTGACCCGG TCGTGCCCCT CTCTAGAGAT AATGAGCATT
      GCATGTCTAA GTTATAAAAA
1301  ATTACCACAT ATTTTTTTTG TCACACTTGT TTGAAGTGCA
      GTTTATCTAT CTTTATACAT ATATTTAAAC TTTAATCTAC
      GAATAATATA ATCTATAGTA
1401  CTACAATAAT ATCAGTGTTT TAGAGAATCA TATAAATGAA
      CAGTTAGACA TGGTCTAAAG GACAATTGAG TATTTTGACA
      ACAGGACTCT ACAGTTTTAT
1501  CTTTTTAGTG TGCATGTGTT CTCCTTTTTT TTTGCAAATA
      GCTTCACCTA TATAATACTT CATCCATTTT ATTAGTACAT
      CCATTTAGGG TTTAGGGTTA
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FIG 8B CONT.

```
1601 ATGGTTTTTA TAGACTAATT TTTTTAGTAC ATCTATTTTA
     TTCTATTTTA GCCTCTAAAT TAAGAAAACT AAAACTCTAT
     TTTAGTTTTT TTATTTAATA
1701 ATTTAGATAT AAAATAGAAT AAAATAAAGT GACTAAAAAT
     TAAACAAATA CCCTTTAAGA AATTAAAAAA ACTAAGGAAA
     CATTTTTCTT GTTTCGAGTA
1801 GATAATGCCA GCCTGTTAAA CGCCGTCGAC GAGTCTAACG
     GACACCAACC AGCGAACCAG CAGCGTCGCG TCGGGCCAAG
     CGAAGCAGAC GGCACGGCAT
1901 CTCTGTCGCT GCCTCTGGAC CCCTCTCGAG AGTTCCGCTC
     CACCGTTGGA CTTGCTCCGC TGTCGGCATC CAGAAATTGC
     GTGGCGGAGC GGCAGACGTG
2001 AGCCGGCACG GCAGGCGGCC TCCTCCTCCT CTCACGGCAC
     GGCAGCTACG GGGGATTCCT TTCCCACCGC TCCTTCGCTT
     TCCCTTCCTC GCCCGCCGTA
2101 ATAAATAGAC ACCCCCTCCA CACCCTCTTT CCCCAACCTC
     GTGTTGTTCG GAGCGCACAC ACACACAACC AGATCTCCCC
     CAAATCCACC CGTCGGCACC
2201 TCCGCTTCAA GGTACGCCGC TCGTCCTCCC CCCCCCCCCC
     TCTCTACCTT CTCTAGATCG GCGTTCCGGT CCATGCATGG
     TTAGGGCCCG GTAGTTCTAC
2301 TTCTGTTCAT GTTTGTGTTA GATCCGTGTT TGTGTTAGAT
     CCGTGCTGCT AGCGTTCGTA CACGGATGCG ACCTGTACGT
     CAGACACGTT CTGATTGCTA
2401 ACTTGCCAGT GTTTCTCTTT GGGGAATCCT GGGATGGCTC
     TAGCCGTTCC GCAGACGGGA TCGATTTCAT GATTTTTTTT
     GTTTCGTTGC ATAGGGTTTG
2501 GTTTGCCCTT TTCCTTTATT TCAATATATG CCGTGCACTT
     GTTTGTCGGG TCATCTTTTC ATGCTTTTTT TTGTCTTGGT
     TGTGATGATG TGGTCTGGTT
2601 GGGCGGTCGT TCTAGATCGG AGTAGAATTC TGTTTCAAAC
     TACCTGGTGG ATTTATTAAT TTTGGATCTG TATGTGTGTG
     CCATACATAT TCATAGTTAC
2701 GAATTGAAGA TGATGGATGG AAATATCGAT CTAGGATAGG
     TATACATGTT GATGCGGGTT TTACTGATGC ATATACAGAG
     ATGCTTTTTG TTCGCTTGGT
2801 TGTGATGATG TGGTGTGGTT GGGCGGTCGT TCATTCGTTC
     TAGATCGGAG TAGAATACTG TTTCAAACTA CCTGGTGTAT
     TTATTAATTT TGGAACTGTA
2901 TGTGTGTGTC ATACATCTTC ATAGTTACGA GTTTAAGATG
     GATGGAAATA TCGATCTAGG ATAGGTATAC ATGTTGATGT
     GGGTTTTACT GATGCATATA
3001 CATGATGGCA TATGCAGCAT CTATTCATAT GCTCTAACCT
     TGAGTACCTA TCTATTATAA TAAACAAGTA TGTTTTATAA
     TTATTTTGAT CTTGATATAC
3101 TTGGATGATG GCATATGCAG CAGCTATATG TGGATTTTTT
     TAGCCCTGCC TTCATACGCT ATTTATTTGC TTGGTACTGT
     TTCTTTTGTC GATGCTCACC
```

FIG 8B CONT.

NcoI
FseI

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3201 CTGTTGTTTG GTGTTACTTC TGCAGGGTAC CCCCGGGGTC
GACCATGGTG ATGAGACGCT ACAAGCTCTT TCTCATGTTC
TGTATGGCCG GCCTGTGCCT
3301 CATCTCCTTC CTGCACTTCT TCAAGACCCT GTCCTATGTC
ACCTTCCCCC GAGAACTGGC CTCCCTCAGC CCTAACCTGG
TGTCCAGCTT TTTCTGGAAC
3401 AATGCCCCGG TCACGCCCCA GGCCAGCCCC GAGCCAGGAG
GCCCTGACCT GCTGCGTACC CCACTCTACT CCCACTCGCC
CCTGCTGCAG CCGCTGCCGC
SacI
~~~~~~~
3501 CCAGCAAGGC GGCCGAGGAG CTCCACCGGG TGGACTTGGT
GCTGCCCGAG GACACCACCG AGTATTTCGT GCGCACCAAG
GCCGGCGGCG TCTGCTTCAA
3601 ACCCGGCACC AAGATGCTGG AGAGGCCCCC CCCGGGACGG
CCGGAGGAGA AGCCTGAGGG GGCCAACGGC TCCTCGGCCC
GGCGGCCACC CCGGTACCTC
3701 CTGAGCGCCC GGGAGCGCAC GGGGGGCCGA GGCGCCCGGC
GCAAGTGGGT GGAGTGCGTG TGCCTGCCCG GCTGGCACGG
ACCCAGCTGC GGCGTGCCCA
3801 CTGTGGTGCA GTACTCCAAC CTGCCCACCA AGGAGCGGCT
GGTGCCCAGG GAGGTGCCGC GCCGCGTCAT CAACGCCATC
AACGTCAACC ACGAGTTCGA

NotI

~~~~~~~~~

3901 CCTGCTGGAC GTGCGCTTCC ACGAGCTGGG CGACGTGGTG
GACGCCTTTG TGGTGTGCGA GTCCAACTTC ACGGCTTATG
GGGAGCCGCG GCCGCTCAAG
4001 TTCCGGGAGA TGCTGACCAA TGGCACCTTC GAGTACATCC
GCCACAAGGT GCTCTATGTC TTCCTGGACC ACTTCCCGCC
CGGCGGCCGG CAGGACGGCT
4101 GGATCGCCGA CGACTACCTG CGCACCTTCC TCACCCAGGA
CGGCGTCTCG CGGCTGCGCA ACCTGCGGCC CGACGACGTC
TTCATCATTG ACGATGCGGA
4201 CGAGATCCCG GCCCGTGACG GCGTCCTTTT CCTCAAGCTC
TACGATGGCT GGACCGAGCC CTTCGCCTTC CACATGCGCA
AGTCGCTCTA CGGCTTCTTC
4301 TGGAAGCAGC CGGGCACCCT GGAGGTGGTG TCAGGCTGCA
CGGTGGACAT GCTGCAGGCA GTGTATGGGC TGGACGGCAT
CCGCCTGCGC CGCCGCCAGT

FIG 8B CONT.

```
4401 ACTACACCAT GCCCAACTTC AGACAGTATG AGAACCGCAC
     CGGCCACATC CTGGTGCAGT GGTCGCTGGG CAGCCCCCTG
     CACTTCGCCG GCTGGCACTG
4501 CTCCTGGTGC TTCACGCCCG AGGGCATCTA CTTCAAGCTC
     GTGTCCGCCC AGAATGGCGA CTTCCCACGC TGGGGTGACT
     ACGAGGACAA GCGGGACCTG
4601 AACTACATCC GCGGCCTGAT CCGCACCGGG GGCTGGTTCG
     ACGGCACGCA GCAGGAGTAC CCGCCTGCAG ACCCCAGCGA
     GCACATGTAT GCGCCCAAGT
4701 ACCTGCTGAA GAACTACGAC CGGTTCCACT ACCTGCTGGA
     CAACCCCTAC CAGGAGCCCA GGAGCACGGC GGCGGGCGGG
     TGGCGCCACA GGGGTCCCGA

     BamHI        PmeI

     ~~~~~~~    ~~~~~~~~~

4801 GGGAAGGCCG CCCGCCCGGG GCAAACTGGA CGAGGCGGAA
     GTCGAACAAA AACTCATCTC AGAAGAGGAT CTGAATTAGG
     ATCCTAGGTT TAAACTGAGG
4901 GCACTGAAGT CGCTTGATGT GCTGAATTGT TTGTGATGTT
     GGTGGCGTAT TTTGTTTAAA TAAGTAAGCA TGGCTGTGAT
     TTTATCATAT GATCGATCTT
5001 TGGGGTTTTA TTTAACACAT TGTAAAATGT GTATCTATTA
     ATAACTCAAT GTATAAGATG TGTTCATTCT TCGGTTGCCA
     TAGATCTGCT TATTTGACCT
5101 GTGATGTTTT GACTCCAAAA ACCAAAATCA CAACTCAATA
     AACTCATGGA ATATGTCCAC CTGTTTCTTG AAGAGTTCAT
     CTACCATTCC AGTTGGCATT

     FseI

     ~~~~~~~~

5201 TATCAGTGTT GCAGCGGCGC TGTGCTTTGT AACATAACAA
     TTGTTCACGG CATATATCCA CGGCCGGCCT AGCTAGCCAC
     GGTGGCCAGA TCCACTAGGG
                    PacI
                  ~~~~~~~~~

                              SwaI
                            ~~~~~~~~

            NotI                      PmeI
     HindIII
         ~~~~~~~~~                  ~~~~~~~~~

     ~~~~~~~

5301 GCAAGCGGCC GCTTAATTAA ATTTAAATGT TTAAACTAGG
     AAATCCAAGC TTGGGCTGCA GGTCAATCCC ATTGCTTTTG
     AAGCAGCTCA ACATTGATCT
```

FIG 8B CONT.

```
5401  CTTTCTCGAG GTCATTCATA TGCTTGAGAA GAGAGTCGGG
      ATAGTCCAAA ATAAAACAAA GGTAAGATTA CCTGGTCAAA
      AGTGAAAACA TCAGTTAAAA
5501  GGTGGTATAA AGTAAAATAT CGGTAATAAA AGGTGGCCCA
      AAGTGAAATT TACTCTTTTC TACTATTATA AAAATTGAGG
      ATGTTTTTGT CGGTACTTTG
5601  ATACGTCATT TTTGTATGAA TTGGTTTTTA AGTTTATTCG
      CTTTTGGAAA TGCATATCTG TATTTGAGTC GGGTTTTAAG
      TTCGTTTGCT TTTGTAAATA
5701  CAGAGGGATT TGTATAAGAA ATATCTTTAA AAAAACCCAT
      ATGCTAATTT GACATAATTT TTGAGAAAAA TATATATTCA
      GGCGAATTCT CACAATGAAC
5801  AATAATAAGA TTAAAATAGC TTTCCCCCGT TGCAGCGCAT
      GGGTATTTTT TCTAGTAAAA ATAAAAGATA AACTTAGACT
      CAAAACATTT ACAAAAACAA
5901  CCCCTAAAGT TCCTAAAGCC CAAAGTGCTA TCCACGATCC
      ATAGCAAGCC CAGCCCAACC CAACCCAACC CAACCCACCC
      CAGTCCAGCC AACTGGACAA
6001  TAGTCTCCAC ACCCCCCCAC TATCACCGTG AGTTGTCCGC
      ACGCACCGCA CGTCTCGCAG CCAAAAAAAA AAAAAGAAAG
      AAAAAAAAGA AAAAGAAAAA

      FseI

      ~~~~~~~~~

6101  ACAGCAGGTG GGTCCGGGTC GTGGGGGCCG GAAACGCGAG
      GAGGATCGCG AGCCAGCGAC GAGGCCGGCC CTCCCTCCGC
      TTCCAAAGAA ACGCCCCCCA
6201  TCGCCACTAT ATACATACCC CCCCCTCTCC TCCCATCCCC
      CCAACCCTAC CACCACCACC ACCACCACCT CCACCTCCTC
      CCCCCTCGCT GCCGGACGAC
6301  GCCTCCCCCC TCCCCCTCCG CCGCCGCCGC GCCGGTAACC
      ACCCCGCCCC TCTCCTCTTT CTTTCTCCGT TTTTTTTTTC
      CGTCTCGGTC TCGATCTTTG

      BamHI

      ~~~~

6401  GCCTTGGTAG TTTGGGTGGG CGAGAGGCGG CTTCGTGCGC
      GCCCAGATCG GTGCGCGGGA GGGGCGGGAT CTCGCGGCTG
      GGGCTCTCGC CGGCGTGGAT
      BamHI
      ~~
6501  CCGGCCCGGA TCTCGCGGGG AATGGGGCTC TCGGATGTAG
      ATCTGCGATC CGCCGTTGTT GGGGGAGATG ATGGGGGGTT
      TAAAATTTCC GCCATGCTAA
```

FIG 8B CONT.

```
6601 ACAAGATCAG GAAGAGGGGA AAAGGGCACT ATGGTTTATA
     TTTTTATATA TTTCTGCTGC TTCGTCAGGC TTAGATGTGC
     TAGATCTTTC TTTCTTCTTT
6701 TTGTGGGTAG AATTTGAATC CCTCAGCATT GTTCATCGGT
     AGTTTTTCTT TTCATGATTT GTGACAAATG CAGCCTCGTG
     CGGAGCTTTT TTGTAGGTAG
       NcoI
      ~~~~~~
6801 ACCATGGCTT CTCCGGAGAG GAGACCAGTT GAGATTAGGC
     CAGCTACAGC AGCTGATATG GCCGCGGTTT GTGATATCGT
     TAACCATTAC ATTGAGACGT
6901 CTACAGTGAA CTTTAGGACA GAGCCACAAA CACCACAAGA
     GTGGATTGAT GATCTAGAGA GGTTGCAAGA TAGATACCCT
     TGGTTGGTTG CTGAGGTTGA
7001 GGGTGTTGTG GCTGGTATTG CTTACGCTGG GCCCTGGAAG
     GCTAGGAACG CTTACGATTG GACAGTTGAG AGTACTGTTT
     ACGTGTCACA TAGGCATCAA
                  BamHI
                  ~~~~~~
7101 AGGTTGGGCC TAGGATCCAC ATTGTACACA CATTTGCTTA
     AGTCTATGGA GGCGCAAGGT TTTAAGTCTG TGGTTGCTGT
     TATAGGCCTT CCAAACGATC
7201 CATCTGTTAG GTTGCATGAG GCTTTGGGAT ACACAGCCCG
     GGGTACATTG CGCGCAGCTG GATACAAGCA TGGTGGATGG
     CATGATGTTG GTTTTTGGCA

     SacI

     ~~~~~~~
7301 AAGGGATTTT GAGTTGCCAG CTCCTCCAAG GCCAGTTAGG
     CCAGTTACCC AGATCTGAGG TACCCTGAGC TCGGTCGCAG
     CGTGTGCGTG TCCGTCGTAC
             FseI
           ~~~~~~~~~
7401 GTTCTGGCCG GCCGGGCCTT GGGCGCGCGA TCAGAAGCGT
     TGCGTTGGCG TGTGTGTGCT TCTGGTTTGC TTTAATTTTA
     CCAAGTTTGT TTCAAGGTGG
7501 ATCGCGTGGT CAAGGCCCGT GTGCTTTAAA GACCCACCGG
     CACTGGCAGT GAGTGTTGCT GCTTGTGTAG GCTTTGGTAC
     GTATGGGCTT TATTTGCTTC
7601 TGGATGTTGT GTACTACTTG GGTTTGTTGA ATTATTATGA
     GCAGTTGCGT ATTGTAATTC AGCTGGGCTA CCTGGACATT
     GTTATGTATT AATAAATGCT

     PmeI

     ~~~~~~~~~
```

FIG 8B CONT.

7701 TTGCTTTCTT CTAAAGATCT TTAAGTGCTG AATTCATATT
TCCTCCTGCA GGGTTTAAAC TTGCCGTGGC CTATTTTCAG
AAGAATTCCC AATAGTAGTC
7801 CAAAATTTTT GTAACGAAGG GAGCATAATA GTTACATGCA
AAGGAAAACT GCCATTCTTT AGAGGGGATG CTTGTTTAAG
AACAAAAAAT ATATCACTTT
7901 CTTTTGTTCC AAGTCATTGC GTATTTTTTT AAAAATATTT
GTTCCTTCGT ATATTTCGAG CTTCAATCAC TTTATGGTTC
TTTGTATTCT GGCTTTGCTG
8001 TAAATCGTAG CTAACCTTCT TCCTAGCAGA AATTATTAAT
ACTTGGGATA TTTTTTTAGA ATCAAGTAAA TTACATATTA
CCACCACATC GAGCTGCTTT
8101 TAAATTCATA TTACAGCCAT ATAGGCTTGA TTCATTTTGC
AAAATTTCCA GGATATTGAC AACGTTAACT TAATAATATC
TTGAAATATT AAAGCTATTA
8201 TGATTAGGGG TGCAAATGGA CCGAGTTGGT TCGGTTTATA
TCAAAATCAA ACCAAACCAA CTATATCGGT TTGGATTGGT
TCGGTTTTGC CGGGTTTTCA
8301 GCATTTTCTG GTTTTTTTTT TGTTAGATGA ATATTATTTT
AATCTTACTT TGTCAAATTT TTGATAAGTA AATATATGTG
TTAGTAAAAA TTAATTTTTT
8401 TTACAAACAT ATGATCTATT AAAATATTCT TATAGGAGAA
TTTTCTTAAT AACACATGAT ATTTATTTAT TTTAGTCGTT
TGACTAATTT TTCGTTGATG
8501 TACACTTTCA AAGTTAACCA AATTTAGTAA TTAAGTATAA
AAATCAATAT GATACCTAAA TAATGATATG TTCTATTTAA
TTTTAAATTA TCGAAATTTC
8601 ACTTCAAATT CGAAAAAGAT ATATAAGAAT TTTGATAGAT
TTTGACATAT GAATATGGAA GAACAAAGAG ATTGACGCAT
TTTAGTAACA CTTGATAAGA
8701 AAGTGATCGT ACAACCAATT ATTTAAAGTT AATAAAAATG
GAGCACTTCA TATTTAACGA AATATTACAT GCCAGAAGAG
TCGCAAATAT TTCTAGATAT
8801 TTTTTAAAGA AAATTCTATA AAAAGTCTTA AAGGCATATA
TATAAAAACT ATATATTTAT ATTTTGGTTT GGTTCGAATT
TGTTTTACTC AATACCAAAC
8901 TAAATTAGAC CAAATATAAT TGGGTTTTTA ATCGCGGCCC
ACTAGTCACC GGTGTAGCTT GGCGTAATCA TGGTCATAGC
TGTTTCCTGT GTGAAATTGT
9001 TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA
TAAAGTGTAA AGCCTGGGGT GCCTAATGAG TGAGCTAACT
CACATTAATT GCGTTGCGCT
9101 CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT
GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG
CGTATTGGGC GCTCTTCCGC
9201 TGCGCACGCT GCGCACGCTG CGCACGCTTC CTCGCTCACT
GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
CAGCTCACTC AAAGGCGGTA

FIG 8B CONT.

9301 ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA
ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA
AAAGGCCGCG TTGCTGGCGT
9401 TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA
TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA
TAAAGATACC AGGCGTTTCC
9501 CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCCTG
CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
GCGTGGCGCT TTCTCATAGC
9601 TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTCGCT
CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA
CCGCTGCGCC TTATCCGGTA
9701 ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC
GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG
AGGTATGTAG GCGGTGCTAC
9801 AGAGTTCTTG AAGTGGTGGC CTAACTACGG CTACACTAGA
AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
CCTTCGGAAA AAGAGTTGGT
9901 AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG
GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA
AGGATCTCAA GAAGATCCTT
10001 TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA
CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG
ATCTTCACCT AGATCCTTTT
10101 AAATTAAAAA TGAAGTTTTA AATCAATCTA AAGTATATAT
GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
AGGCACCTAT CTCAGCGATC
10201 TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTCG
TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC
CAGTGCTGCA ATGATACCGC
10301 GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA
CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA
ACTTTATCCG CCTCCATCCA
10401 GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG
CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG
GCATCGTGGT GTCACGCTCG
10501 TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT
CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC
GGTTAGCTCC TTCGGTCCTC
10601 CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTTATCACT
CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG
CCATCCGTAA GATGCTTTTC
10701 TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG
TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAATAC
GGGATAATAC CGCGCCACAT
10801 AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT
CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC
CAGTTCGATG TAACCCACTC

FIG 8B CONT.

```
10901  GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG
       CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA
       AAAAAGGGAA TAAGGGCGAC
11001  ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT
       TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT
       ACATATTTGA ATGTATTTAG
11101  AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA
       AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC
       ATTAACCTAT AAAAATAGGC
11201  GTATCACGAG GCCCTTTCGT CTCGCGCGTT TCGGTGATGA
       CGGTGAAAAC CTCTGACACA TGCAGCTCCC GGAGACGGTC
       ACAGCTTGTC TGTAAGCGGA
11301  TGCCGGGAGC AGACAAGCCC GTCAGGGCGC GTCAGCGGGT
       GTTGGCGGGT GTCGGGGCTG GCTTAACTAT GCGGCATCAG
       AGCAGATTGT ACTGAGAGTG
11401  CACCATATGC GGTGTGAAAT ACCGCACAGA TGCGTAAGGA
       GAAAATACCG CATCAGGCGC CATTCGCCAT TCAGGCTGCG
       CAACTGTTGG GAAGGGCGAT
11501  CGGTGCGGGC CTCTTCGCTA TTACGCCAGC TGGCGAAAGG
       GGGATGTGCT GCAAGGCGAT TAAGTTGGGT AACGCCAGGG
       TTTTCCCAGT CACGACGTTG
11601  TAAAACGACG GCCAGTGAAT TACACCGGTG TGATCATGGG CCG
```

FIG 8B CONT.

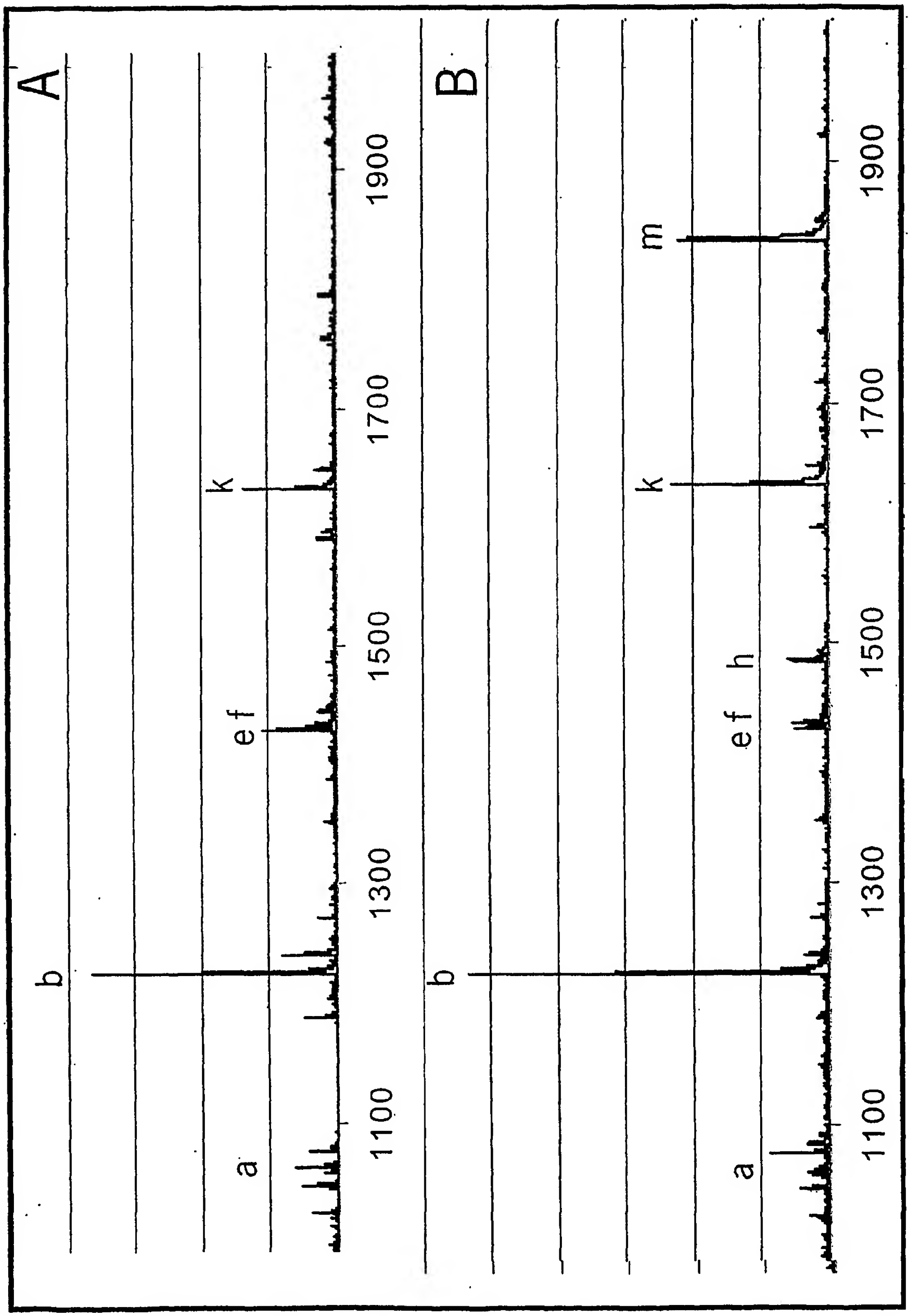
A
b
a
e f
k
1100
1300
1500
1700
1900
B
b
a
e f
h
k
m
1100
1300
1500
1700
1900

FIG. 9

atgaagatgagacgctacaagctctttctcatgttctgtatggccggcctgtgcctcatctccttcctgcacttcttcaagaccct
gtcctatgtcaccttcccccgagaactggcctccctcagccctaacctggtgtccagctttttctggaacaatgccccggtca
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accggccacatcctggtgcagtggtcgctgggcagcccccctgcacttcgccggctggcactgctcctggtgcttcacgcc
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ctgaactacatccgcggcctgatccgcaccgggggctggttcgacggcacgcagcaggagtacccgcctgcagacccc
agcgagcacatgtatgcgcccaagtacctgctgaagaactacgaccggttccactacctgctggacaacccctaccagga
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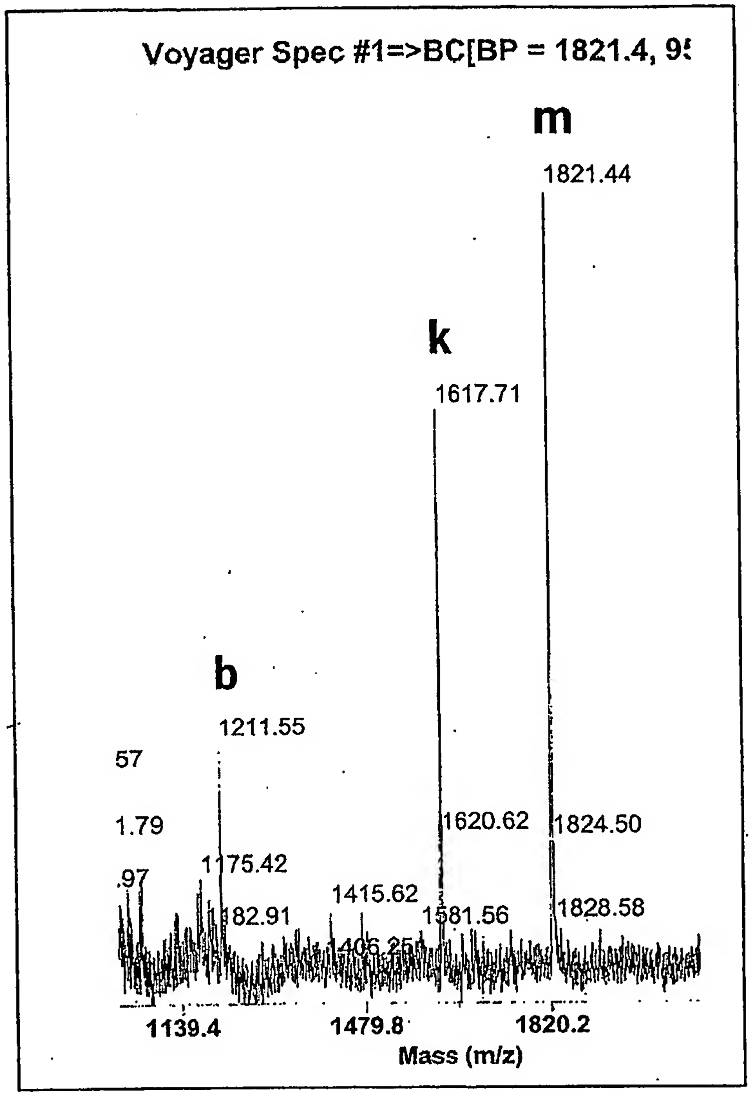
acgaggcggaagtctag

FIG. 10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1581.56
1824.50
1828.58
1139.4
1479.8
1820.2
Mass (m/z)

FIG 11

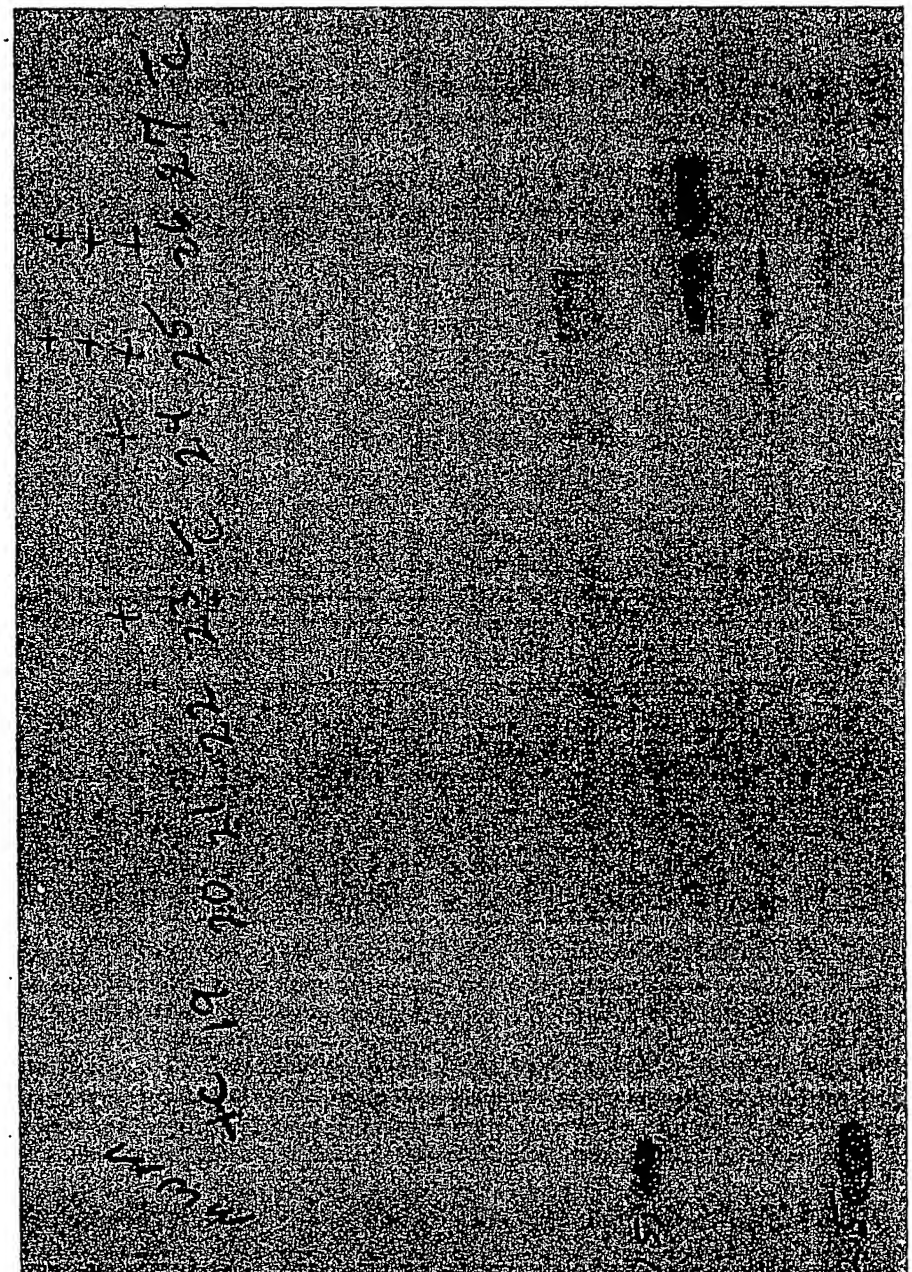


FIG 12

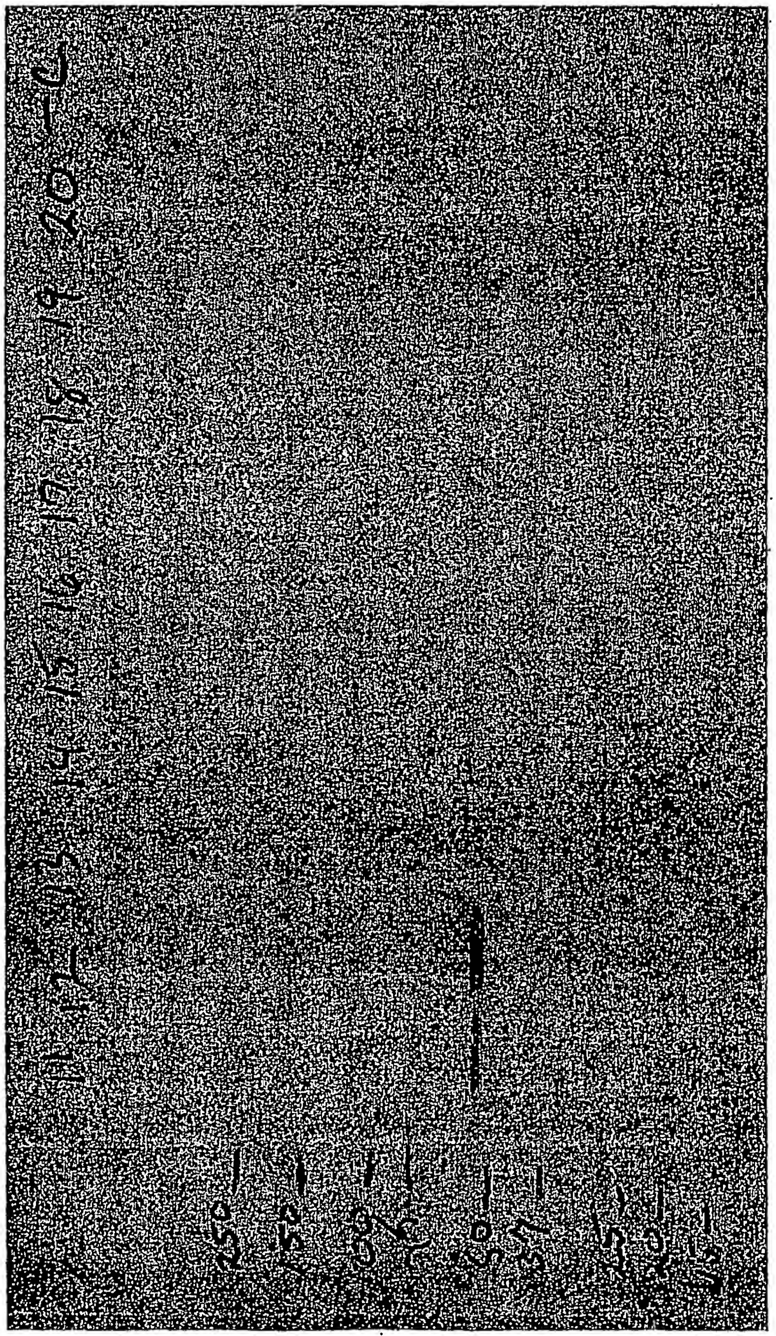
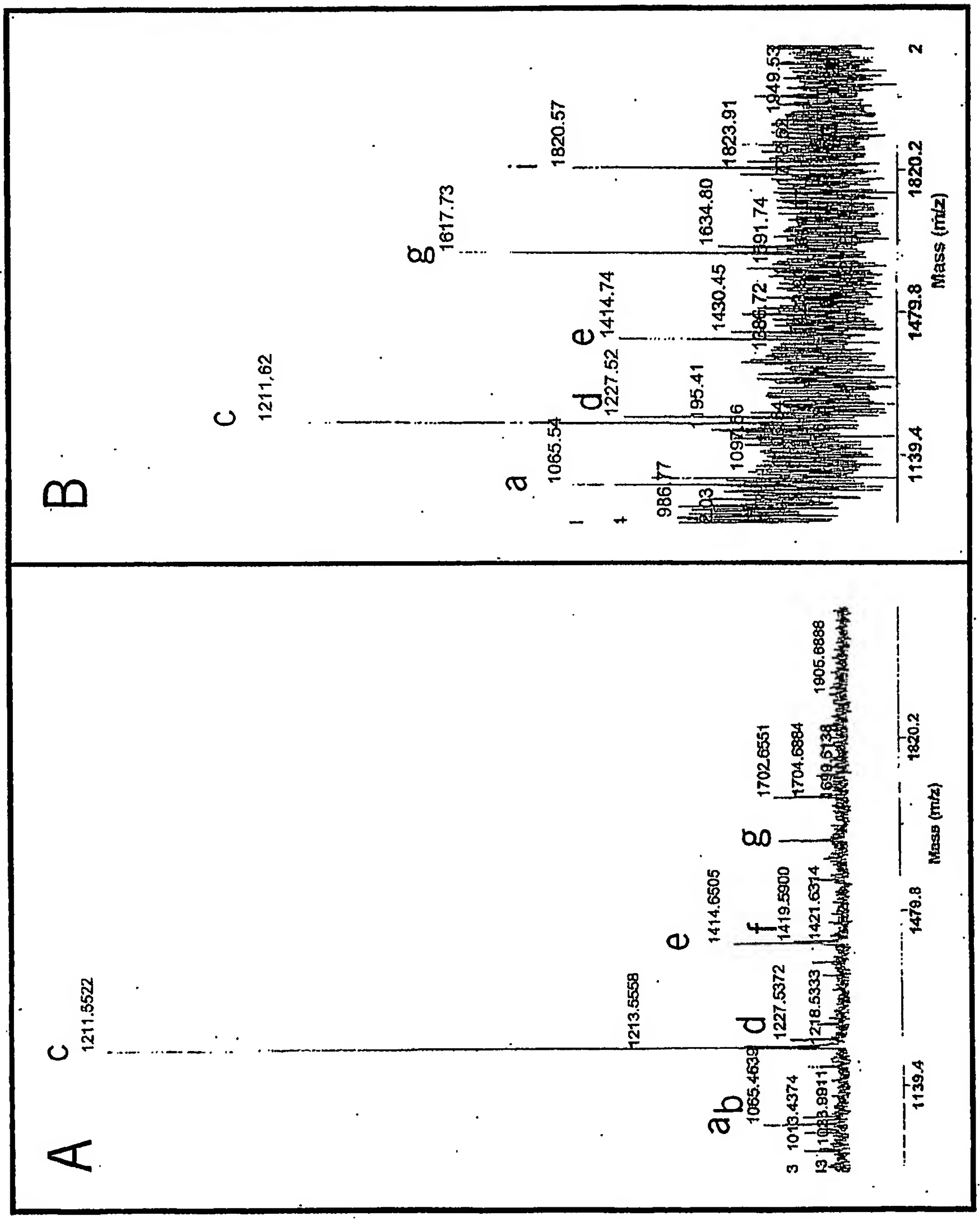


FIG 13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a b
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e
1414.6505
f
1419.5900
1421.6314
g
1702.6551
1704.6884
1699.6138
1905.6888
1139.4
1479.8
1820.2
Mass (m/z)
B
a
1065.54
986.77
c
1211.62
d
1227.52
1195.41
1097.56
e
1414.74
1430.45
g
1617.73
1634.80
1591.74
i
1820.57
1823.91
1949.53
1139.4
1479.8
1820.2
Mass (m/z)

FIG. 14

SEQUENCE LISTING

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BAKKER, Hendrikus A.C.
FLORACK, Dionisius E.A.
BOSCH, Hendrik J.

<120> GNTIII expression in plants

<130> 62862A - P03331300

<150> US-60/365,769
<151> 2002-03-19

<150> US-60/368,047
<151> 2002-03-26

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